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STUDIES ON THE RELATIONSHIPS BETWEEN EARTHWORMS AND SOIL FERTILITY

I. BIOLOGICAL STUDIES IN THE FIELD

BY A. C. EVANS AND W. J. MCL. GUILD

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(With 10 Text-figures)

Changes in the seasonal activity of several species of earthworms have been followed in a permanent pasture field for 18 months, 1945–6. The two soil conditions which chiefly determine activity are temperature and moisture. Other factors are the occurrence of an obligatory diapause in the two species *Allolobophora nocturna* and *A. longa*, and changes in population in *A. chlorotica* and *Lumbricus terrestris*.

Soil temperature and soil moisture also determined the weight of soil thrown up in the form of wormcasts during autumn, winter and spring. It is suggested that only two species, *A. nocturna* and *A. longa*, are responsible for wormcasts and that the other four common species present play little or no part in this activity.

At Rothamsted it was found that the previous agricultural history of the field is an important factor in determining the fauna. Old permanent pasture is characterized by a high percentage of *A. nocturna* and a rather lower percentage of *A. caliginosa*. Ploughing old permanent pasture and reseeding to grass after 1 or 2 years arable reduces the proportions of *A. nocturna* and *A. caliginosa* and increases that of *Eisenia rosea*. Arable fields have *A. chlorotica* as the dominant species and pasture fields 2–7 years grass after many years of arable farming still show a high percentage of *A. chlorotica* and a low proportion of *A. nocturna*.

A survey of the Carse of Stirling, Scotland, showed that soil type is also an important factor in determining the earthworm fauna. *A. caliginosa* was the dominant species on the three soil types studied, but the subdominant species varied. On clay soil, *A. longa* was subdominant; on loam, *A. longa* and *L. rubellus*; on sandy soil, *A. longa*, *L. rubellus* and *A. chlorotica*. The dominant species of pasture land at Rothamsted, *A. nocturna*, was not found at Stirling.

INTRODUCTION

The relationships between bacteria, protozoa and soil fertility have been well studied for many years, but scarcely any work has been carried out on the effects on soil fertility of the numerous invertebrates present. Since earthworms are present in most soils of agricultural importance, consume dead organic matter of varying degrees of decomposition, and pass large quantities of soil through their bodies in the course of a year, it was decided to carry out an intensive programme of research on their importance for soil fertility.

In spite of their natural abundance in soils of widely varying types and their supposed value in the economy of nature, extremely little is known of their biology. So, in order to plan experiments on their effects on soil fertility and to interpret the results of these experiments, detailed biological studies were found necessary. This

work falls naturally into two parts: the determination of the length of the life cycle and rate of reproduction of the various species, and the seasonal variation in their activities in the field. This paper is concerned with the latter part of the programme, as the life-history studies are not yet complete and will be reported on later.

Techniques

Two independent techniques were used for studying seasonal activities: the collection of the casts thrown up on the surface of grassland, previously used by several investigators, and the collection of earthworms brought to the surface by the use of a vermifuge. The observations were carried out over a period of 2 years on Great Field III, a pasture field which has been down to grass for at least 70 years. For the collection of wormcasts, a number of circular plots 7 sq.ft. in area, evenly distributed over the field, were marked out. For the season August 1944 to March 1945, ten plots were marked out and the casts collected twice a week; for the season April 1945 to May 1946, twelve plots were marked out and the casts collected every fourth day. The number of casts and the weight of the casts, dried at 105° C., was recorded for each plot.

A number of substances have been used in greenkeeping as earthworm expellents in order to prevent the formation of wormcasts on golf-greens, bowling-greens, etc. If these substances should bring up a representative sample of the various species present in the soil, their value for survey work on the effect of soil type, previous history of the field, etc. on the composition of the earthworm fauna would be very great. Dawson, Boyns & Shorrock (1938) tested solutions of potassium permanganate at various concentrations and compared them with Mowrah meal and found that concentrations of $\frac{1}{4}$ and $\frac{1}{2}$ oz. of potassium permanganate per gal. per sq.yd. were as efficient as the standard rate of Mowrah meal. It was therefore decided to test the use of potassium permanganate for survey work. Each of ten plots of grassland, $2{\cdot}7 \times 2{\cdot}7$ ft., was treated with $\frac{1}{4}$ oz. of potassium permanganate dissolved in 1 gal. water. The worms which came to the surface were dropped into 4% formalin immediately as otherwise they rapidly disintegrated into an unidentifiable mass. After the worms had ceased coming up, the areas were dug over and all the worms found preserved for identification. In all, 149 adults of all species were obtained by watering with potassium permanganate and thirty-four by digging; 941 immature worms were obtained by watering but none by digging. The soil was so wet after watering that any remaining small worms would have been extremely difficult to find. The proportions of the different species found are given in Table 1.

The results show that under the soil conditions at the time of sampling a high recovery of the total population was obtained and also that the proportion of the different species brought up by the potassium permanganate solution was representative of that of the population as a whole. An advantage of this technique is that the worms do not retreat into the subsoil as they may do when a fork or spade is driven into the ground.

Later work at various times of the year suggested that the proportion of the total population brought up by potassium permanganate was conditioned by the soil temperature and soil moisture conditions at the time of sampling. So it was decided in March 1945 to commence regular sampling by this method to test the effect of soil and seasonal factors on the number of worms brought up and the proportions of the different species present. Ten samples were taken at each observation. This sampling was carried out on Great Field III so that any changes in earthworm activity could be correlated with the production of wormcasts. Weekly estimations, at least, of the soil moisture were carried out and the soil temperature records of the Meteorological Station, situated in an adjoining field, were used.

The faunas of the various fields are compared on a basis of the adult and nearly adult individuals present, because the specific characters of the Lumbricidae are essentially secondary sexual characters and only appear when the individual is nearly full grown. A technique has been devised recently by which it is sometimes

TABLE 1

	Percentage composition of the earthworm fauna obtained by		
	Permanganate	Digging	Total
A. nocturna	12·1	11·8	12·0
A. caliginosa	59·0	47·0	56·8
A. chlorotica	4·7	5·9	4·9
E. rosea	20·0	35·3	22·9
L. terrestris	4·0	0·0	3·3

possible to identify a large proportion of the immature individuals present, providing the adult fauna of the field from which the sample is taken is known accurately. The chief diagnostic character is the number of segments. Other useful characteristics are colour, size and the form assumed on preserving in dilute formalin (6–8%). For instance, *Allolobophora chlorotica* contracts and presents a stubby appearance but *Eisenia rosea* relaxes and dies in a loose coil with its dorsal pores wide open. The chief factor which interferes with identifications by this method is the loss of segments due to bird damage, but this is minimized in the case of quite small worms since the bird is more likely to capture the whole rather than the part. In the fauna at Rothamsted this technique of identification has proved quite practicable except for *A. nocturna* and *A. longa*. The adults of these two species have a mean segment number of 220 and 175 and standard deviations of 25 and 18 respectively so that there is a considerable overlap. All except two of the species of Lumbricidae examined so far have approximately the adult number of segments when the worm emerges from the cocoon. The two exceptions are *A. nocturna* and *A. longa* which have approximately 150 segments at hatching. This makes the separation of the two species in the immature stages impossible by using the segment number technique, and in view of the close similarity of the adults, there is little or no hope of separating them in the immature stages.

Seasonal changes in the activity of earthworms

Fig. 1 shows the seasonal changes in the total number of worms from March 1945 to June 1946. Soon after the observations were begun a dry spell set in and the total number of worms per ten samples fell from just over 300 to five. In spite of a considerable increase in soil moisture during the early part of May the number of worms only increased slightly and during June, July and August never rose above 100. The autumn rains commenced early in September and the soil moisture increased from 17 to 28%, and there was a corresponding increase in the number of worms from nil on 28 August to 601 on 18 September. The number of worms fell rapidly during a dry spell which occurred during the first 3 weeks of October and again rose rapidly when rain fell, but the numbers did not regain their previous maximum. By this time the soil temperature at 4 in. had fallen considerably and it can be seen that the fluctuations in numbers from November to March closely follow the changes in soil temperature. During this period the soil moisture remained high and any effects of the slight changes were swamped by the greater changes in soil temperature. During a remarkably dry March and April (1946) the soil moisture fell from 47 to 18% and again the numbers of worms fell correspondingly. The numbers increased during the very wet spell which occurred in June but did not approach the numbers expected under the soil conditions occurring at the time. An explanation of this will be offered below.

A statistical analysis of the results given in Fig. 1 was carried out by the method of multiple covariance in groups (Snedecor, 1946), for the data obtained between March 1945 and May 1946. The data were divided into seven groups of six readings. By using this method, it was hoped that the effects of any big changes in the actual population would be eliminated. For analysis the data were transformed to the logarithmic scale as follows:

$$t = \log (\text{soil temperature } ^\circ \text{F.} - 30),$$
$$m = \log (\% \text{ soil moisture content} - 15),$$
$$w = \log (\text{number of worms per ten plots} + 1).$$

The results of the analysis showed that there were no significant differences between the group totals, F for six and thirty-three degrees of freedom falling just short of significance. The significance of the partial correlation coefficients $r\,wt.m = 0{\cdot}3982$ and $r\,wm.t = 0{\cdot}3744$ was tested by means of the multiple correlation coefficient $R = 0{\cdot}4936$ which is significant at the 1% level.

It was rather surprising that no significant differences could be detected between the group totals because such differences almost certainly exist. During the summer periods *Allolobophora longa* Ude, *A. nocturna* Evans, *A. chlorotica* Sav., *A. caliginosa* Sav. and *Eisenia rosea* Sav. spend a great deal of time in a state of diapause, rolled up in a tight ball in a spherical earthen cell. The two former species appear to undergo an obligatory diapause for several months during the summer as previously suggested by Avel (1929) for *A. terrestris* Sav. and *A. longa*. These two species and *A. nocturna*

are very closely related. *A. chlorotica*, *A. caliginosa* and *E. rosea*, however, undergo a facultative diapause, only rolling up into a ball when soil conditions are unfavourable and becoming active when soil conditions are favourable again. On the other hand, no evidence has been found during these field investigations that *Lumbricus terrestris* goes into a diapause. This species has been found to be active all through the year and when the surface soil is very dry has been found active in the moist subsoil at depths of 4–6 ft. Thus, during the dry summer of 1945, five out of six commonly found species were in a state of diapause and so not in a condition to react to the potassium permanganate. The reason for not finding significant differences between the group totals lies in the high variability of the observations and the fact that regression only accounts for 24% of the total variance. The coefficient of variation of the original data is high, 37·6%, and is only reduced to 32·7% when the variance due to regression is eliminated.

The changes in the seasonal activity of the different species are extremely interesting. Although only a small proportion of the total population can be identified with certainty, i.e. adults or individuals nearly adult with the tubercula pubertatis but not the clitellum clearly differentiated, sufficient numbers of the common species were obtained to warrant a statistical examination of the results by the method of multiple covariance in groups. Few adult *L. terrestris* were obtained, but since the only other species present, *L. castaneus* Sav., which could be confused with it in the immature state is present in very small numbers, the analysis was carried out on the total number of *Lumbricus* spp. captured.

Fig. 2 shows the changes in activity of five species from August 1945 to June 1946. There are clear responses to changes in soil temperature and soil moisture in all species. With the advent of the autumn rain all species increase rapidly but decrease during a short dry spell during early October. They all increase again when the soil moisture increases but to different degrees compared with their initial peaks. By this time falling temperature has a noticeable effect. Three species, *L. terrestris*, *Allolobophora caliginosa* and *Eisenia rosea*, increase to numbers nearly equal to their initial peak. On the other hand, *A. chlorotica* does not reach to even 50% of its initial peak, but *A. nocturna* far outstrips it and reaches its maximum activity during November. There is a general reduction in the activity of all the species during the winter, but warmer spells are accompanied by increased activity.

There would appear to be a very definite reduction in the actual population of *A. chlorotica* since, even during warm spells, the number captured never approached that obtained during the autumn. During the cold spell at the end of January 1946 the temperature at 4 in. under grass dropped to 31·9° F. and no worms were brought up by the permanganate. As the soil temperature increased during February all species showed an increase in numbers but the general level of activity in spring did not reach that attained in the autumn. This was probably due to the checking effect of a dry spell during March and April before the soil temperature had become sufficiently high for maximum activity. By the time the wet spell of May had

sufficiently moistened the soil for full activity, varying proportions of the different species were in facultative or obligatory diapause.

This series of observations was planned to finish on 28 May 1946, the same date as the wormcast observations, as by that date approximately 10% of the area under examination would have been sampled. In view of the continued wet spell during June, two more sets of samples were taken to see whether those species classified as having a facultative diapause would break the diapause and become active again. The numbers of the three species tentatively classified as possessing a facultative diapause increased as a result of the increased soil moisture but to varying degrees. *Eisenia rosea* increased considerably in numbers compared with the general level of activity throughout the series of observations, *Allolobophora chlorotica* increased compared with its spring level but the increase in *A. caliginosa* was very slight. Increases in both sexually mature worms and 'adolescent' worms occurred for *E. rosea*, but for *A. caliginosa* and *A. chlorotica* the increase was almost solely confined to 'adolescent' worms. Unfortunately, no information is available as to the length of the life-history of these species, nor in fact of any of the earthworms, either under controlled laboratory conditions or in the field. This prevents a detailed interpretation of field results.

The numbers of the adults of the two species *A. nocturna* and *A. longa*, which appear to have an obligatory diapause, did not increase as a result of increased soil moisture in May and June. However, fairly large numbers, about 70% of the number present during the adult peak of November 1945, of large immature specimens recognizably belonging to one or other of these species were present. It may be that the obligatory diapause only occurs in individuals which have reached sexual maturity. This is supported by observations on about fifty specimens of these two species which are being reared from the time of hatching in separate containers and have shown no sign of an obligatory diapause during the period of sexual immaturity. On the other hand, adults of these species, kept under observation in optimum conditions of food, soil moisture and temperature do show an obligatory diapause in summer. Avel (1929) also found that it occurred during his extensive observations on the adults of *A. terrestris* and *A. longa*.

The results of the statistical analyses on the correlation between activity of the five common species, expressed as numbers captured, and soil temperature and soil moisture, are given in Table 2.

Both partial correlation coefficients are significant or highly significant for cases of *L. terrestris*, *A. nocturna* and *A. caliginosa* but for *E. rosea* only the partial correlation of activity on moisture is significant. In *A. chlorotica* neither coefficient is significant although both approach significance. It is difficult to believe that the activity of *A. chlorotica* is not governed to some extent by soil temperature and moisture, and it is thought that significance has not been shown in this series of observations because of the large overriding effect of the progressive decline in the real population.

The regression coefficients of activity on temperature are greater than those on moisture except in the case of *E. rosea* where they are equal. Both coefficients are greater for *L. terrestris* than for the other four species, which show smaller differences.

The group totals adjusted to mean soil temperature and moisture, by means of the regression formulae, are illustrated in Fig. 3. The population of *A. chlorotica* remains constant during September and November and then declines considerably, very few specimens indeed being taken during April and May. It is thought that these figures illustrate a real decline in the adult population since no specimens in a state of diapause have been found during digging operations in winter or early spring. The population of *A. caliginosa* remains fairly constant from October to March. The lower numbers found in September and May are probably due, in September, to a lag effect of the increased soil moisture on the worms coming out of diapause and,

TABLE 2. *Partial correlation coefficients of five species of earthworms (log transformation of data)*

w = activity, t = temperature, m = moisture

	$rwt.m$	$rwm.t$	r for 22 degrees of freedom
A. nocturna	0·5560	0·5265	P 0·05 = 0·488
A. caliginosa	0·6076	0·4896	P 0·01 = 0·585
A. chlorotica	0·4485	0·4680	
E. rosea	0·4188	0·5437	
L. terrestris	0·7180	0·7023	

TABLE 3. *Regression coefficients of the activity of five species of earthworms on temperature and moisture (log transformation of data)*

	Temperature	Moisture
A. nocturna	1·44	0·81
A. caliginosa	1·48	0·65
A. chlorotica	1·07	0·68
E. rosea	0·68	0·70
A. terrestris	2·28	1·32

in May, to the worms going into diapause as a result of low soil moisture. There would appear to be a real increase in the adult population of *A. nocturna* during October since the peak population is not reached until November. This late peak cannot be caused by any lag effect of emergence from diapause since 100% of the specimens of this species captured by digging in October were active. No significant changes in adult population were found for *E. rosea* while *L. terrestris* showed an increase in the total population from September to October after which the population remained constant.

During these observations, the relative proportions of the adults of the different species active at any one time showed very considerable seasonal differences (Fig. 4). These differences are evidently due to a number of factors already discussed above, namely, presence or absence of diapause (obligatory or facultative), changes in actual

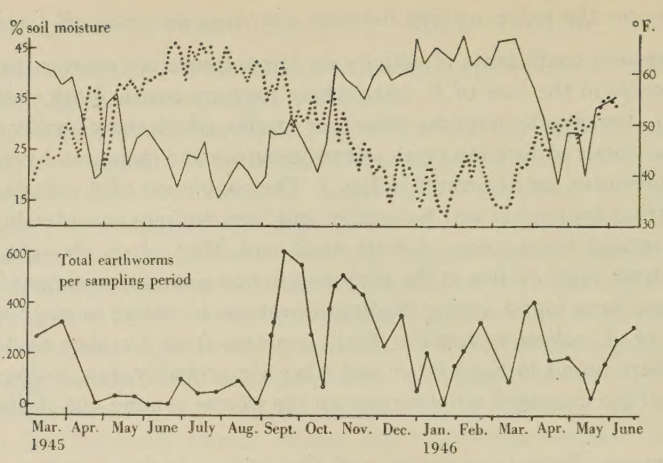
% soil moisture
°F.
Total earthworms per sampling period
Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June
1945
1946

Fig 1

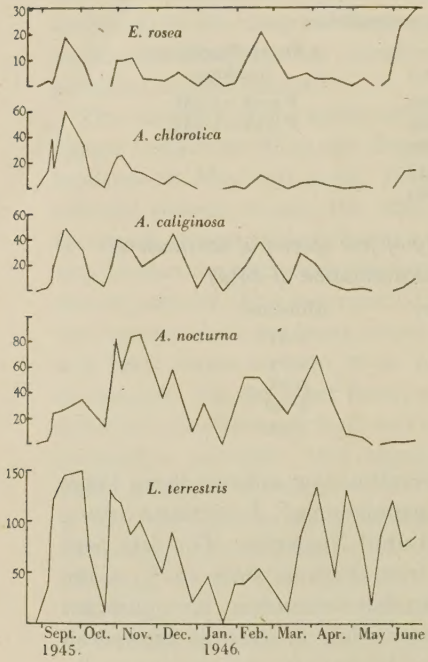

Fig. 2

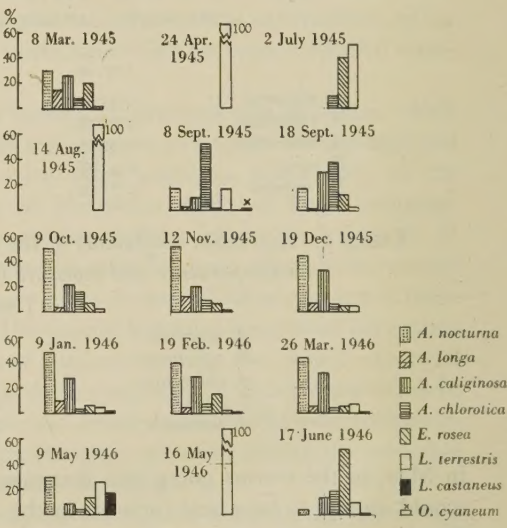

Fig. 4

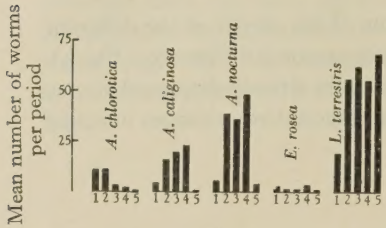

Fig. 3

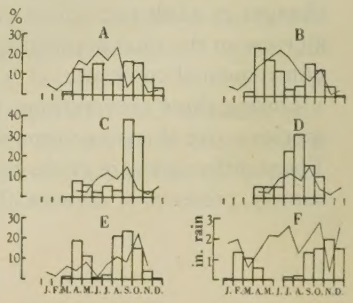

Fig. 5

population and varying regression coefficients of activity on soil moisture and temperature. In early March 1945, *A. nocturna*, *A. caliginosa* and *E. rosea* were the dominant species; these same species were also dominant in late February 1946. When very dry soil conditions were occurring in late April 1945 only a few specimens were obtained. These were adult and immature individuals of *L. terrestris* which remained the dominant active species until the beginning of August, only a few adult and immature specimens of the other species being obtained. In September, *A. chlorotica* was dominant but its relative abundance rapidly declined and not until June 1946 was it relatively abundant again. From October 1945 until March 1946, *A. nocturna* was dominant with *A. caliginosa* subdominant, the other four species being present but in relatively low numbers. *A. longa*, *L. castaneus* and *Octolasium cyaneum* Sav. were never present in large numbers and never formed the larger proportion of the population. In early May 1946 *A. nocturna* was just dominant with *E. rosea*, *L. terrestris* and *L. castaneus* constituting a higher proportion of the population than they had done during the previous autumn and winter. In late May, during dry conditions, only *L. terrestris* was active, but during the wet spell of June *E. rosea* was the dominant species with *A. caliginosa*, *A. chlorotica* and *L. terrestris* subdominant and *A. nocturna* and *L. castaneus* just represented.

The total weight of the worms (after preservation in formalin) collected at each sampling was determined to obtain an approximation of the weight of worms per acre on Great Field III. In general, the curve of the weight of worms per sample closely followed that of numbers per sample but the maximum weight, 40 g. per sample, was recorded 2 weeks after the maximum number per sample. This was largely due to the larger numbers of *A. nocturna* taken per sample. The mean weight of the six heaviest samples was 35 g. corresponding to 4·3 cwt./acre. A loss of approximately 20% occurred due to immersion in formalin. Applying a correction for this, and bearing in mind that even under favourable conditions all the worms in

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Legends to Text-figs. 1–5

Fig. 1. The seasonal changes in activity of all earthworms with the corresponding changes in soil moisture at 2 in. and temperature at 4 in. from March 1945 to June 1946. —— percentage soil moisture at 2 in.; ..... soil temperature ° F. at 4 in.; –●– total earthworms per ten samples.

Fig. 2. The seasonal changes in activity of five species of earthworms, *E. rosea*, *A. chlorotica*, *A. caliginosa*, *A. nocturna* and *L. terrestris*.

Fig. 3. The seasonal changes in population, after eliminating the effects of soil moisture and soil temperature, of five species of earthworms. 1, 28 Aug.–2 Oct.; 2, 9 Oct.–12 Nov.; 3, 20 Nov.–21 Jan.; 4, 29 Jan.–2 Apr.; 5, 13 Apr.–28 May.

Fig. 4. Relative changes in the proportions of the different species active from 8 March 1945 to 17 June 1946. (Adult and nearly adult specimens.)

Fig. 5. The seasonal changes in wormcast production expressed as a percentage of the annual total production for six different habitats. The monthly rainfall is also shown. A, golf-course, Zurich; B, wooded meadow, Nieder-Gosgen; C, steppe-heath, Bellinchen; D, oakwood, Bellinchen; E, grass (park), Breslau; F, permanent pasture, Rothamsted.

the treated area do not come to the surface, it can be concluded that the weight of earthworms on Great Field III is of the order of $6\frac{1}{2}$ cwt./acre.

The production of wormcasts under widely differing climatic conditions

It was Darwin (1837) who first studied the production of wormcasts and drew attention to its role in the formation of the surface layer of soil, varying in depth up to about 6–7 in. and called by him 'vegetable mould'. The weight of soil thrown up on the surface during a year in the form of castings was measured in two areas and estimated in two others by Darwin (1881). Where the casts were regularly collected over a period of just over a year, they amounted to 7·6 and 16·1 tons/acre. The estimated amounts were 14·6 and 18·1 tons/acre. These latter figures are probably rather higher than the average for the fields sampled as they were admittedly collected from areas where worms or wormcasts 'abounded'. Three other investigators, Stockli (1929), Dreidax (1931) and Kollmansperger (1934), have also studied the production of wormcasts in various habitats for periods of a year or more, but again one or two areas (1 sq.m.) only were studied in each habitat so again no estimation can be made of the average production of wormcasts over a large area. A selection from the results of the above investigators, together with data obtained at Rothamsted, is given in Table 4 in order to show the wide variation which occurs.

Table 4. *Comparison of amounts of soil thrown up per annum by earthworms in different habitats*

Description of habitat	*p*H	Tons/acre
(1) Old turf, sandy soil. Common land	—	16·1
(2) Old turf, sandy soil	—	7·5
(3) Steppe-heath, marly loam	8·1	2·1
(4) Felled beechwood, marly loam	7·3	2·7
(5) Oakwood, sandy soil	7·6	2·3
(6) Garden soil, clay loam	7·2	3·6
(7) Wooded meadow, sandy loam	7·1	31·2
(8) Permanent meadow, clay loam	6·9	12·2
(9) Golf course (edge), clay loam	6·3	32·4
(10) Mixed wood, clay loam	5·7	8·1
(11) Grass (in a park) (good soil)	6·9	36·5
(12) Arable, under wheat, sandy loam	6·2	9·9
(13) Arable, fallow, sandy loam	6·3	3·5
(14) Permanent pasture, clay with flints 1944–5	—	11·5
(15) Permanent pasture, clay with flints 1945–6	—	11·0
(16) Valley of the White Nile, Sudan	—	107·0

(1) and (2) after Darwin, (3)–(5) after Kollmannsperger, (6)–(10) after Stockli, (11)–(13) after Dreidax, (14) and (15) present investigations, (16) after Beaugé.

The amount of soil thrown up during the year varies considerably according to the habitat. The smallest amounts recorded are from the Bellinchen region on the Oder, by Kollmannsperger (1934). This region is hot with an annual rainfall of 19 in. falling mainly from May to September, not sufficient to moisten the soil to field capacity. In the areas studied, the organic matter content of the soil was low.

Higher amounts are recorded from soils near Zurich by Stockli (1929). The lowest amount recorded was on a garden soil, 3–6 tons/acre and the highest, 32·4 tons/acre, on the edge of an hotel golf course. An amount nearly as great as the maximum was recorded on a wooded meadow which is manured every year with liquid manure; intermediate amounts, varying from 8·1 to 12·2 tons/acre, were recorded from several other habitats of grassland and woodland types. The soils in this series of observations were apparently much more favourable for worm activity than those at Bellinchen, having a higher organic matter content; they were also rather more acid. In 1924, the year in which the observations were made, the rainfall was approximately 37 in. At Breslau, 36·5 tons/acre were recorded from a grass area in a public park but only a quarter of that amount on an arable field under wheat. On a portion of the wheat field which was being fallowed still less was recorded, only 3·5 tons/acre. Dreidax attributes this decrease to an altered soil climate, the fallow soil being exposed to greater contrasts of baking sun and beating rain than the soil under the wheat crop. The rainfall at Breslau was approximately 32 in. The records for England are of an order similar to the intermediate values for Switzerland. The rainfall for the 2 years of observation at Rothamsted was 25·8 and 28·8 in. No doubt there are areas in England where more than 16 tons/acre of wormcasts are thrown up. One such area is situated in a Yorkshire dale, Fryupdale. One of us (A. C. E.) visited this area at the end of September 1945 and took samples of the worm population in areas where the casting was high and where it was more normal. In the areas where casting was high, the occupiers of the two farms most affected stated that in October, certain portions of the pastures resemble a ploughed field so great is the production of wormcasts (locally termed worm-sprouts). This also occurs in arable fields and the casting under stooks at harvest time may reach 1–2 ft. up into the stooks. The highest record of wormcasts production to date is 107 tons/acre, observed in the valley of the White Nile in the Sudan (Beaugé, 1912). This quantity was thrown up during the 6 months of the rainy period.

Not only does the total annual weight of wormcasts vary considerably from place to place, but also the time of year at which production chiefly occurs. Fig. 5 shows the seasonal trends in wormcast production, expressed as a percentage of the annual total, for four different regions in Europe and four different habitats. The seasonal trend of the rainfall is also given. The five diagrams representing continental conditions have one point in common, namely, the total absence of wormcast production in the months of January and February. This is in contrast to conditions at Rothamsted where in January a small amount, and in February 1945 a considerable amount, of casting occurred. In January 1946 more casting occurred than in January 1945. The production of wormcasts is also greater in December at Rothamsted than on the Continent.

These contrasts are closely related to the winter soil temperatures, which are lower on the Continent than in England. In Switzerland at Zurich the level of wormcast production was almost uniform during spring, summer and autumn, but at Nieder-

Gosgen (26 miles away) there was a distinct drop during July and August although the rainfall at the two stations was very similar. At Bellinchen, on Steppe heath, maximum production occurred during September and October, but in an oak-wood nearby it occurred during July and August. At Breslau, as at Nieder-Gosgen and Rothamsted, there was a distinct diminution in the production of casts during summer. The greatest diminution of cast production in summer occurred at Rothamsted during May to August. The depression in cast production during summer would appear to be partly due to diminished rainfall and increased rate of loss of water from the soil at Breslau and Rothamsted and perhaps at Nieder-Gosgen. Unfortunately, the data given by the various investigators are not sufficient to permit of an adequate explanation of these seasonal changes. For instance, Stockli (1929) studied ten different habitats for 1 or 2 years but made no attempt to estimate the numbers of worms in the different habitats; in fact, in a comprehensive study of the effect of earthworms on various aspects of the soil, he never once mentions an earthworm by a specific or generic name.

Seasonal changes in wormcast production at Rothamsted

From August 1944 to May 1945 the collection of wormcasts from the plots was made twice every week. In practice this meant that alternate collections represented the amounts thrown up during periods of 3 days and 4 nights, and 4 days and 3 nights. Since most of the casts seem to be thrown up during the night the two collection periods are not equally balanced and so the collections for the year are figured in Fig. 6 as weekly collections. In the succeeding 12 months the collections were carried out every fourth day, a period of time which allowed a reasonable amount to be accumulated but not long enough for the casts to be broken down by wind and rain.

The autumn rain began on 18 August and the first collection was made on 31 August. The numbers and weight of casts increased steadily to a maximum of sixty-four casts weighing 132 g./plot per week at the end of October. The rainfall was spread fairly evenly over September and October. By the beginning of November the soil temperature had fallen by 10° F. and now became the limiting factor. Continued decrease in temperature was accompanied by decrease in the amount of casts until an increase in temperature occurred at the end of November. This was accompanied by a marked increase in casting. Low soil temperatures occurred throughout January and casting was at a minimum. Increase in soil temperature during February 1945 was accompanied by a rapid increase in casting which reached its spring maximum in the middle of February, but this maximum was only about two-thirds that of the autumn maximum. From this time onwards the amount of casting fell at a uniform rate although the soil temperature and moisture during late February and early March appeared favourable for its maintenance. The estimation of soil moisture was begun on 5 March 1946. April was

a dry month with only 0·3 in. of rain between the first and twenty-fifth day, the soil moisture falling from 38 to 20%. During the last 5 days of April and first 7 days of May 1·8 in. of rain fell with a consequent increase in soil moisture to 35%, but there was no corresponding increase in casting, which continued falling until the middle of May when, except for a few instances, it virtually ceased until August. At the time it was considered that this was due to all the earthworms entering into aestivation. This explanation was not correct as will be shown below.

Speaking generally, soil temperatures and soil moisture changed fairly steadily during the period August 1945 to April 1946 and the changes in the amount of casting were also steady. In contrast to this the period July 1945 to May 1946 was characterized by two spells of dry weather, in October 1945 and March–April 1946, months which usually have a fair amount of rain. Soil temperatures were also irregular between December and March. These changes are very clearly reflected in the amount of casting (Fig. 7). The October dry spell reduced the soil moisture from 34 to 19% and the amount of casting per 4-day period from 60 to 15 g./plot. At the end of October the maximum amount of casting for the autumn was recorded, 80 g./plot. This weight was nearly reached again during warm spells in December and February. The close correspondence between fluctuations in the amount of casting and fluctuations in soil temperature and moisture show that these two soil factors have a big influence on the amount of wormcasts thrown up on to the surface.

A statistical examination of the data was carried out by the method of multiple covariance in groups on a logarithmic transformation of the data.

TABLE 5. *Analysis of* 1945–6 *wormcast data,* 112 *observations divided into sixteen groups*

	Correlation coefficients			
	Numbers		Weights	
	Total	Within groups	Total	Within groups
Weight	0·9933	—	—	—
Temperature	−0·2114	0·4819	−0·1596	0·5160
Moisture	0·4790	0·4717	0·4849	0·4681
Temperature and moisture: Total			−0·4622	
Within groups			0·0905	

$P = 0{\cdot}01$. $r = 0{\cdot}297$, D.F. (total) 110; $r = 0{\cdot}312$, D.F. (within groups) 90.

Of the total correlations four are significant, namely, numbers and weights of casts, soil moisture with both numbers and weights of casts and soil temperature with soil moisture. Since it has been shown above that there are probably seasonal changes in the basic population of earthworms the relevant information is contained in the 'within groups' columns. Thus the correlations between numbers of wormcasts with soil temperature and soil moisture, and also between weights of wormcasts

with the same two soil factors are very highly significant. The partial correlation coefficients are as follows:

	Numbers	Weights
Moisture independent of temperature	0·4906	0·4940
Temperature independent of moisture	0·5002	0·5382

All are very highly significant.

From the data necessary to calculate the correlation coefficients it is possible to work out formulae relating numbers and weights of casts to soil temperature and moisture independent of seasonal trends and then to use the formulae to adjust the group means to see whether any seasonal trends occur. The regression formulae are as follows:

$$\text{Number of wormcasts} = (T-32)^{0\cdot8624} \times (M-15)^{0\cdot8801} \times 0\cdot5912$$
$$\text{Weight of wormcasts} = (T-32)^{1\cdot0054} \times (M-15)^{0\cdot9083} \times 0\cdot4773$$

T = soil temperature in ° F. M = % soil moisture.

Since the variance of both T and M are very similar, 5·83 and 5·66, and the regression coefficients within each formula are similar, it is evident that temperature and moisture are of approximately equal potency in estimating numbers and weights of casts. The group-means adjusted to mean temperature and moisture are shown in Fig. 8. It is clear that there is a very definite seasonal trend, independent of soil temperature and moisture, casting being very low in summer, high in autumn, lower in winter followed by a spring increase and summer decrease. This curve strongly resembles that of the seasonal activity of the earthworm population, particularly that of *A. nocturna* which has a period of obligatory diapause in summer.

Forty samples were taken between March 1945 and May 1946 to study the seasonal activity of earthworms. The figures obtained have been used to correlate the activity of the total earthworm activity and the activity of portions of the total population with numbers and weights of casting, temperature and moisture. The relevant correlation coefficients are given in Table 6. The numbers and weights of casts are correlated with the numbers of worms active (total worms) at a highly significant level.

Table 6. *Total correlation coefficients*

	Total worms	*A. nocturna*	Other species	Weight of casts	No. of casts	Temperature
Weight of casts	0·6653	0·8576	0·5455	—	—	—
Number of casts	0·5688	0·8155	0·4472	0·9723	—	—
Temperature	0·3296	0·0005	0·3612	−0·1752	−0·1701	—
Moisture	0·4897	0·5172	0·4216	0·3481	0·3984	−0·4978

$r = 0\cdot304$, $P = 0\cdot05$. $r = 0\cdot393$, $P = 0\cdot01$.

It has been tacitly assumed in the literature that all earthworms void some of their faeces on to the surface of consolidated soil. Experience with breeding the different species in pots suggested that only two (*A. nocturna* and *A. longa*) out of the nine species bred voided their faeces to any extent on to the surface of the soil. This

supposition was strengthened to some extent by field experience, particularly in May and June 1946. During these 2 months the amount of casting was very low, being almost nil in June although soil conditions of temperature and moisture were very favourable for casting and the total number of worms active was only slightly less than half of the autumn maximum (Figs. 1 and 7). Of the worms active in June the two species suspected of being chiefly responsible for casting formed only a very low proportion, and those present were mostly young immature specimens. From the correlation coefficients given in Table 6 it is possible to calculate a number of partial correlation coefficients to check the hypothesis outlined above. Unfortunately, it is only possible, for this purpose, to divide the total worms into two groups, *A. nocturna* and 'other species', since the proportion of *A. longa* in the population is extremely low. The partial correlation coefficients between numbers and weights of casts with *A. nocturna* independent of 'other species' are 0·8327 and 0·8231 ($P = < 0{\cdot}01$), and the corresponding coefficients with 'other species' independent of *A. nocturna* are −0·5171 and −0·3912. Since it is not likely biologically that the numbers and weights of casts are diminished by the presence of other species, it may be concluded that, in the present circumstances, *A. nocturna* is the chief agent responsible for the production of wormcasts in Great Field III. The significant negative correlations between 'other species' and casting are probably due to the fact that these other species are active at periods when little or no casting occurs, i.e. in summer when *A. nocturna* and *A. longa* are in a state of obligatory diapause.

With the data in Table 6 we can go further and test whether soil temperature and moisture influence casting only through their effect on the number of *A. nocturna* active or whether they have additional independent effects, i.e. on the ease with which soil is consumed at various soil moistures. This can be done by means of the multiple correlation coefficient R (Snedecor, 1946, p. 362). In the case of *A. nocturna*, temperature and moisture $R = 0{\cdot}9031$ for weight of wormcasts and 0·8476 for numbers of wormcasts. Since the total correlation coefficients for *A. nocturna* with weights and numbers of wormcasts are 0·8576 and 0·8155, both slightly lower than the corresponding R's, it would seem that soil temperature and soil moisture influence casting only through their influence on worm activity. This conclusion can be checked by calculating the partial correlation coefficients for temperature and moisture with numbers and weights independent of *A. nocturna*. All four coefficients are non-significant, their values being as follows:

	No.	Weight of casts
Temperature	−0·2932	−0·2050
Moisture	−0·0468	−0·2172

The total cast production of the individual plots showed great variation, certain plots always producing a high quantity of casts and others a low quantity under favourable soil conditions. An analysis of variance was carried out on 28-day totals for each plot for the season September 1945–May 1946. After eliminating the effect of seasonal changes, F for differences between plots was found to be highly

significant. The position of the high and low cast-producing plots were irregularly scattered about the field and not related to any topographical features. The chief cause of the differences between plots is probably the very irregular distribution of organic matter in the fields. Dunging by the grazing stock causes local patches high in organic matter and this in turn causes patchiness in worm population. Soil samples taken from areas which had obviously had dung dropped on them some months earlier showed a mean population five times higher than samples taken from areas which had not obviously been dunged. The difference in the mean weight of worms per sample was even greater—twelve times.

The range in total cast production per plot was considerable. In the season 1944–5 it was 4·6–16·8 tons/acre with a mean of $11{\cdot}5 \pm 1{\cdot}2$. In the season 1945–6 it was 6·2–16·6 tons/acre with a mean of $11{\cdot}0 \pm 0{\cdot}6$.

During the season 1944–5 the average weights of the wormcasts per collection showed clear seasonal trends, increasing to a maximum (1·4 g.) during November then declining to a low value (0·4 g.) in the middle of January, increasing to the maximum (1·4 g.) in early May when the observations on this particular set of plots terminated. During the season 1945–6 no such regular change in mean weight was recorded, the changes being quite irregular. This difference between the two seasons is probably due to differences in weather conditions; soil temperature and probably also soil moisture changed regularly from September 1944 to May 1945, but during September 1945 to May 1946 the changes in these two factors were very erratic.

Comparative populations on fields of varying agricultural history

Although the method of bringing earthworms to the surface of the soil by means of potassium permanganate does not permit of an estimate of the total population, so long as the sampling is carried out on grassland under similar conditions of soil temperature and moisture, then the number of worms brought to the surface on different fields should provide a comparative estimate of their earthworm population. The survey of the fields of the Rothamsted Farm in spring 1946 was carried out in 3 days during which soil conditions remained very constant. Brief descriptions of the areas sampled are given before discussing the differences in their populations.

Old Grassland:

Great Fields I and III, put down in 1872, usually grazed with sheep and cattle, rarely shut up for hay.

Parklands, very old parkland; until a few years ago with a large number of deciduous trees but these have been gradually cleared during the last 4 years.

Leys and New Grass:

Pastures, seventh year grass after old arable.

Claycroft, fourth year grass, previously larch and spruce, felled in 1939, reclaimed 1942.

Parklands Wedge, third year grass following one year arable, previously permanent grass.

Great Field II, second year grass following 2 years arable, previously permanent grass.

New Zealand, first year grass following 2 years arable, previously grass put down in 1928.

Stackyard, grass put down in 1928, sampled March 1945. This field could not be sampled in 1946 as it had been ploughed up.

Miscellaneous:

Great Harpenden, permanent arable, sampled September 1944.

Knott Wood, young beech plantation, ground flora mainly of grass.

Fryupdale, Yorkshire, permanent grass on heavy clay, sampled September 1945.

TABLE 7. *Mean number of worms per sample (area 7 sq.ft.) from ten samples on fields of varying agricultural history. March* 1946

(1) Parklands	84·6	Significantly greater than (3)–(9)
(2) Pastures	77·4	
(3) Great Field II	53·9	Significantly greater than (7)–(9)
(4) Parklands Wedge	50·1	
(5) Claycroft	46·2	
(6) Great Field III	36·3	Significantly greater than (8)–(9)
(7) Great Field I	27·5	
(8) New Zealand	14·8	—
(9) Beech Plantation	11·2	—
Fryupdale A	160·0	—
Fryupdale B	42·0	—

Standard error ±9·7.

In Table 7 the fields have been grouped according to their similar populations. Parklands, an old permanent pasture, has the highest population, which is not significantly higher than that of Pastures, 7 years grass following many years arable. The next group of three fields have all been under grass for only 2–4 years, Claycroft previously was under larch and spruce, and Parklands Wedge and Great Field II, 1 and 2 years arable after permanent pasture. The two other permanent pastures, Great Fields I and III have comparatively low populations. The lowest populations are found in the young beech plantation and in New Zealand, first year grass after 2 years arable. The mean population per sample of two areas in Fryupdale, Yorkshire are included in Table 7 for general comparison. Although the conditions of soil temperature and moisture were rather different from those of the Rothamsted survey, our general experience of this type of sampling strongly suggests that the population at Fryupdale A, area of heavy casting, is much higher than the highest population recorded at Rothamsted, and that of Fryupdale B, area of light casting, is of the same order as those at Rothamsted.

Factors causing the differences in population on the various fields

The low population on New Zealand is possibly due to a big reduction of the original population during the 2 years of arable cultivation, the field being sampled before any appreciable recovery could take place. Stackyard, an adjoining field with a similar agricultural history, had a mean number of sixty-six worms per sample in February 1945 so that the reduction in population was of the order of 75%. An important fact to be borne in mind when comparing populations of worms is the big differences which exist between the size of the various species. Average weights per individual (in grams) for adults, and populations of four species, are shown below:

	Adults	Population
L. terrestris	4·4	1·1
A. nocturna	2·5	0·8
A. caliginosa	0·7	0·26
A. chlorotica	0·25	0·16

Thus it is possible for one field to have a much higher population than another but for the latter field to carry a much greater weight of earthworms, because its dominant species is much larger than that of the other field. In fact, this is what seems to occur in the present set of observations. For instance, Pastures and Claycroft have much higher populations than Great Fields I and III but the dominant species on Pastures is *A. chlorotica*, a small worm, on Claycroft *A. caliginosa*, a medium-sized worm, but on Great Field I and II *A. nocturna*, a heavy worm, is the dominant species.

A further set of observations will be taken on these fields, taking into account sample and species weight and a more detailed analysis will then be possible.

Legends to Text-figs. 6–10

Fig. 6. The seasonal changes in wormcast production from September 1944 to June 1945. ..... soil temperature ° F. at 4 in.; —— percentage soil moisture at 2 in.

Fig. 7. The seasonal changes in wormcast production from July 1945 to May 1946. ..... soil temperature ° F. at 4 in.; —— percentage soil moisture at 2 in.; ● number of wormcasts per plot.

Fig. 8. The seasonal changes in A, wormcast weights and B, wormcast numbers, after eliminating effects of soil moisture and soil temperature, for 28-day periods from March 1945 to May 1946.

Fig. 9. Relative proportions of the different species on fields of different agricultural history.

Fig. 10. Relative proportions of the different species on permanent pasture fields on different soil types in the Carse of Stirling, Scotland. Legends as in Fig. 9, with : *O. lacteum*, •• *A. icterica*.

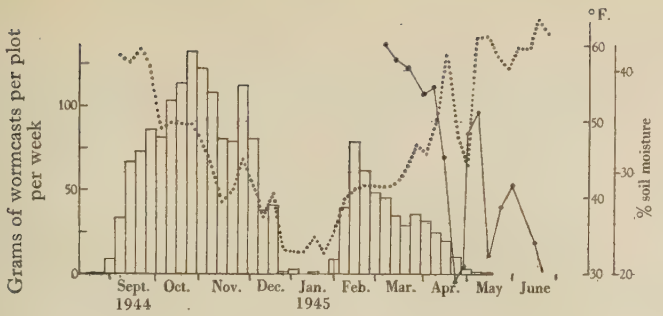

Fig. 6

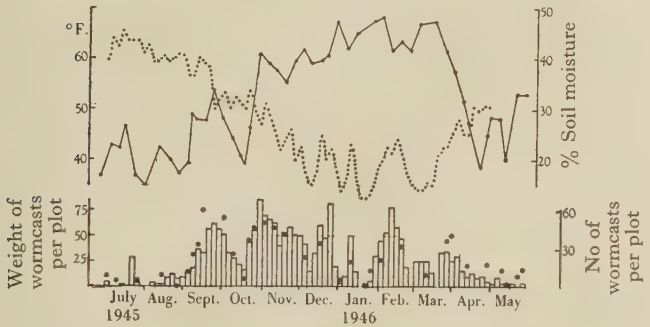

Fig. 7

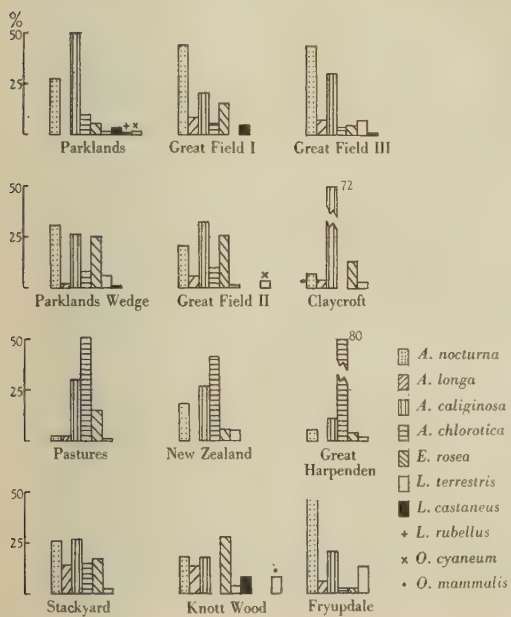

Fig. 9

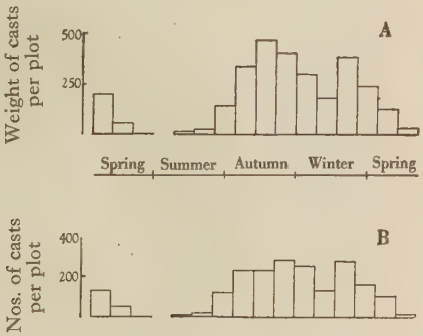

Fig. 8

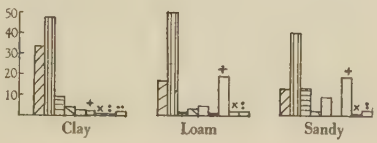

Fig. 10

The distribution of the species in fields of varying history

Little is known of the distribution of the various species of earthworms and how it is affected by the numerous factors likely to be operative in nature. Quantitative faunistic studies of various habitats have been reported by Dreidax (1931), Kollmannsperger (1934), and Bornebusch (1930). The worms were obtained by digging and so it is very possible that some species, particularly *Lumbricus terrestris* and *A. longa*, will not be represented in their true proportions since these species retreat deeply into the subsoil when disturbed. By far the commonest species recorded in the environs of Breslau by Dreidax (1931) was *A. caliginosa*, which occurred in all twelve of the varied habitats sampled; *L. terrestris* occurred in ten, *A. chlorotica* in six and *A. longa* in three. Other species, not named, occurred in small numbers in six habitats. Bornebusch (1930) studied several large samples of soil dug from Danish forests. Again *A. caliginosa* was the commonest species in the three forests studied. Other fairly common species were *A. chlorotica*, *L. rubellus*, *L. castaneus* and *Eisenia rosea*. The least common species were *L. terrestris*, *L. festivus*, *Octolasium cyaneum*, *O. lacteum*, *A. longa* and *Dendrobaena octaedra*. In the investigations carried out by Kollmannsperger (1934) in the Bellinchen region on the Oder, *A. caliginosa* was again by far the most common species, occurring in twenty out of the twenty-three extremely varied habitats sampled, being absent from the two steppe-heath habitats and a water meadow which was flooded for one-third of the year in which the observations were made. *E. rosea* was also very common, occurring in nineteen habitats, *L. rubellus*, *O. lacteum* and *A. chlorotica* were also common, occurring in twelve of the habitats.

The history of the various fields on the Rothamsted farm is known and a study was made of the effect of previous history on the fauna of a series of fields of a reasonably uniform soil type. Also a long series of samples was taken in October 1945 in the Stirling area of Scotland, by one of us (W. J. G.). In this region a number of different soil types occur within the area studied, approximately 4 sq. miles. Samples from other regions have also been taken as opportunities occurred.

A comparative study of the fauna on the different fields at Rothamsted was begun in September 1944, and four surveys were carried out by February 1945. The results of the first three surveys were not particularly satisfactory, as with increasing practice in the identification of this difficult group the earlier identifications became suspect. The results did show, however, that the species complex varied greatly from field to field and seemed to be determined to a large extent by the previous agricultural history of the field. It was intended to carry out a survey in October 1945, as the result of the first four surveys suggested that the proportions of the various species present differed somewhat in spring and autumn, *A. chlorotica* being less common in the spring and *E. rosea* more common. Unfortunately the dry spell which occurred in October limited opportunities for field work and the survey was abandoned in favour of other work. Nine fields were sampled on

26–29 March 1946, at a time when the seasonal activity of the worms was at its spring maximum (Fig. 1). Histograms showing the proportions of the different species present on these fields, together with certain others obtained at other times, are given in Fig. 9.

The fields surveyed show a wide variation in their agricultural history (p. 322), and this is reflected in the species complex now present. The four permanent grass fields are characterized by high proportions of *A. nocturna* and *A. caliginosa*, the former being the dominant species in three fields. The other species are present in comparatively low proportions, except that *E. rosea* is fairly abundant in Great Field I and *L. terrestris* at Fryupdale.

The effect of 1 and 2 years arable (cereal crops) cultivation on Parklands Wedge and Great Field II has not made a great difference to the species complex. The dominance of *A. nocturna* and *A. caliginosa* is not so marked as on the permanent grass fields and the proportion of *E. rosea* has risen, the three species being co-dominant. The fields which have not had a long period under permanent grass show quite different proportions of the species present. In the three fields Stackyard, Pastures and New Zealand, the present species complex seems to be determined, to some extent at least, by the fauna present when the fields were arable. The species complex present on a permanent arable field, sampled at the end of September in stubble after winter beans, shows a very high proportion of *A. chlorotica* and low proportions of the four other species. Pastures and New Zealand still show a dominance of this species, with *A. caliginosa* subdominant. In Pastures, which has been under grass for 7 years, the proportion of *A. nocturna* is still extremely low but Stackyard and New Zealand, which were under grass for at least 15 years before the latter was ploughed, show higher proportions of this species, but still not so high as Great Field I, III and Fryupdale. Biological work in progress on the various species suggests that the increase in the proportion of *A. nocturna* from $2\frac{1}{2}\%$ in a field of 7-years grass, to 20–25% in fields of 15-years grass, up to 40–50% in fields of permanent grass may be due to its comparatively low rate of reproduction and of growth compared with *A. chlorotica* and *A. caliginosa*. Claycroft, a field in its fourth year of grass and a previous history of larch and spruce, has a fauna quite distinct from that of any other field sampled: *A. caliginosa* is by far the most numerous species present, *A. nocturna* again being low. Five surveys of this field have been carried out, but only in one of them were any adult *A. chlorotica* found, two specimens being present out of fifty-four adults. In one sample in the survey of 22 November 1944 eighty-three adult *A. caliginosa* were taken, equivalent to half a million per acre. In the young beech plantation, Knott Wood, four species, *A. nocturna*, *A. longa*, *A. caliginosa* and *Eisenia rosea* were equally abundant. It seems to be a feature of the fauna of other parts of the wood that these four species are about equally abundant.

In Fig. 9 *Octolasium cyaneum* is shown as being present in two fields only, Parklands and Great Field II. This species has at one time or the other been

recorded from all the area surveyed at Rothamsted with the exception of New Zealand of which field only one survey has been carried out. It is also present at Fryupdale. There are also notable omissions in the fauna of two areas. *A. longa* appears to be absent in Parklands, not one specimen having been taken out of 400 adults in five surveys or in the many worms dug from the field for biological work: *A. chlorotica* also appears to be absent from the main area of Knott Wood adjacent to Claycroft in which field it is extremely rare. Not one specimen of *O. lacteum*, considered to be a common species elsewhere, has ever been taken on the farm. *Lumbricus rubellus* is another common species of earthworm which is rarely taken at Rothamsted, a few specimens have been taken in parts of Knott Wood and in Parklands, one of the permanent grass fields.

The complete fauna (adults and immatures) of three fields obtained during the survey of March 1946 has been analysed by the segment number technique and the results compared with that obtained from the adults and nearly adult specimens only (Table 8).

Table 8. *Comparison of the adult fauna of three fields with the total fauna (percentages)*

	New Zealand		Pastures		Parklands	
	Adult	Total	Adult	Total	Adult	Total
A. nocturna / *A. longa*	18·4	25·5	5·0	7·4	27·4	36·2
A. caliginosa	27·3	25·5	28·6	17·5	50·6	25·6
A. chlorotica	42·4	34·8	50·4	13·1	9·5	3·5
E. rosea	6·1	10·0	15·1	6·6	6·6	3·5
L. terrestris	6·1	4·3	0·8	55·2	1·2	29·8
L. rubellus	0	0	0	0	0·6	1·0
L. castaneus	0	0	0	0	3·0	0·3
O. cyaneum	0	0	0	0·2	1·2	0·3

Only in the case of New Zealand do the two methods give tolerably comparable results. For Pastures and Parklands the results are widely divergent. In both fields the percentage of *L. terrestris* is very many times greater on the total count than the adult count. This can be explained by the presence in all samples of large numbers of recently hatched individuals of this species, a feature which was also noted on certain other fields during the March 1946 survey. Even allowing for this high percentage of *L. terrestris* the proportions of the other common species show marked differences. In Pastures the adults of *A. chlorotica* were twice as numerous as those of *A. caliginosa* but on a basis of the total individuals *A. chlorotica* was rather less numerous than *A. caliginosa*. In Parklands the adults of *A. caliginosa* were twice as numerous as those of *A. nocturna* but there were about half as many again total individuals of *A. nocturna* than of *A. caliginosa*. These differences suggest that differences in the breeding season, rate of reproduction and death rate of the various species may occur.

During the survey of March 1946 large numbers of recently hatched individuals of *L. terrestris* were noted in several fields. Such an abundance of this species did not occur in any of the previous surveys, one of which was made in March 1945. The following fields were characterized by high percentages (30–55) of *L. terrestris*: Pastures, Knott Wood, Claycroft and Parklands; the other fields had percentages ranging from 4 to 27. No relationship can be traced between the history of the fields and the percentage of *L. terrestris* present. There is, however, a significant negative correlation between the percentages of *A. nocturna* plus *A. longa* with *L. terrestris* $r = -0{\cdot}7414$, $P < 0{\cdot}05$, $> 0{\cdot}01$. These three species are the largest found at Rothamsted and the negative sign of the correlation coefficient suggests that some degree of competition may exist between the two species of *Allolobophora* and *L. terrestris*.

The distribution of the species on fields of different soil types at Stirling

The survey of the Stirling area showed that the earthworm fauna differs strikingly from that at Rothamsted in certain particulars and also that differences occur in the fauna of the several soil types. Fig. 10 shows the proportions of the various species present on permanent pasture on three soil types, clay, loam and sandy soil. On all three soils *A. caliginosa* is the dominant species, accounting for 40–50% of the adults present. On the clay soil, *A. longa* is subdominant with the following species present in low proportions: *A. chlorotica*, *A. icterica*, *E. rosea*, *L. terrestris*, *L. rubellus*, *O. cyaneum* and *O. lacteum*. On the loam, *A. longa* and *L. rubellus* are subdominant with the following species present in low proportions: *A. chlorotica*, *E. rosea*, *L. terrestris*, *L. rubellus*, *L. castaneus*, *O. cyaneum* and *O. lacteum*. On the sandy soil *L. rubellus*, *A. chlorotica* and *A. longa* are subdominant, *L. terrestris* being present in a higher proportion than on the other two soil types, and *E. rosea*, *O. cyaneum* and *O. lacteum* are present in low proportions.

In view of the differences existing in the earthworm fauna of different soil types, the fauna at Rothamsted can only be compared with that at Stirling on permanent pasture on the clay soil. The most striking difference is the absence of *A. nocturna* at Stirling, this species being dominant on permanent pastures at Rothamsted, *A. longa* is present in much greater proportions at Stirling, 25–40%, than at Rothamsted, 0–10%. *L. rubellus* was present on three out of four fields at Stirling but only on one of three at Rothamsted. Several other fields at Rothamsted, Great Field II, Parklands Wedge, Stackyard and New Zealand, which have been under grass for 20 years or more, have been sampled frequently but not one specimen of *L. rubellus* has been found. Six individuals of *A. icterica*, a rare species in Britain, were taken in one field at Stirling. This species has not been recorded on the Rothamsted farm, but a number of individuals have been taken from an allotment in the laboratory ground adjacent to a shrubbery in the garden of the Director, containing shrubs obtained from abroad. *E. rosea*, *A. chlorotica* and *O. cyaneum* are

present in about the same proportions in both areas but several specimens of *O. lacteum* were taken at Stirling. This species has not yet been recorded at Rothamsted.

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AN ECOLOGICAL STUDY OF THE ENCHYTRAEID WORM POPULATION OF SEWAGE BACTERIA BEDS

LABORATORY EXPERIMENTS

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(With 1 Text-figure)

This paper records the results of the experimental phase of an ecological study of the enchytraeid population of the sewage beds of the Huddersfield Corporation. The sewage in this drainage area is heavily charged with chemical wastes which have a marked effect on the fauna. *Enchytraeus albidus* Henle was the dominant worm while *Lumbricillus lineatus* Mull., the common bacteria bed-worm, was relatively scarce. The ability of these two worms to complete their life cycles in the sewage before and after treatment in particular beds was examined.

The crude Huddersfield sewage proved toxic to both *E. albidus* and *L. lineatus* but the former was much more resistant. After treatment in the beds the degree of toxicity was much reduced and the final sewage was harmless to all stages of *E. albidus* but the eggs of *L. lineatus* were still unable to develop in it.

The eggs of both species were less resistant to the toxic substances than the young worms; the adults were most resistant. High temperatures lowered toxicity by reducing the duration of the more susceptible early stages.

There was no evidence of physiological adaptation of *L. lineatus* to conditions in the Huddersfield beds. Wastes from both the main chemical works in the drainage area were examined and shown to be toxic; the domestic sewage was non-toxic. Both the distillate and residue of one of the chemical wastes proved toxic, especially the latter.

The toxicity could be entirely removed from the treated sewage by standing it over activated carbon for a few hours, or permitting slow oxidation for some weeks. The eggs of *L. lineatus* were used for these tests.

The Huddersfield sewage was very acid from time to time and the acidity reached was sufficient to kill young worms and adults of both species. But this factor was not considered important in the beds owing to the persistent effects of the toxic sewage.

Introduction

As Imms (1937) points out, ecological problems are ideally approached by two complementary lines of inquiry, the one a study of field conditions, the other the disentangling of field problems under controlled laboratory conditions. But frequently the two phases interest different groups of biologists, particularly when the broad problem has an applied aspect. This paper describes the laboratory phase of an intensive ecological study of the enchytraeid population of the Huddersfield sewage bacteria beds, a problem with applied interests. The beds have to deal with a sewage containing a high proportion of chemical waste and the fauna is consequently unique for this type of habitat (Golightly, 1940; Reynoldson, 1941). The results of the field observations have already been given (Reynoldson, 1947), but as they provide the problems studied in the laboratory, it is necessary to summarize

briefly the main conclusions. The enchytraeid population was found to be small compared with that of bacteria beds in other drainage areas. *Enchytraeus albidus* Henle, a small white worm of wide natural distribution, but not previously recorded from the bacteria bed, was the dominant species. *Lumbricillus* (=*Pachydrilus*) *lineatus* Mull. a small pink worm also widely found in nature (Stephenson, 1930) and the species usually abundant in most bacteria beds (Reynoldson, 1939*a*), was scarce. Unlike other bacteria beds, in which the enchytraeid population is concentrated immediately below the surface, in the Huddersfield beds it was much lower, occurring at a depth of 1 ft. Three types of bed were studied, two dealing with sewage at different rates, and the third with a sewage already partially treated. Each type was found to harbour a numerically different population. These were the main problems which were tackled in the laboratory.

Neither routine methods of operation nor structural features of the Huddersfield beds seemed to account for the facts noted above. Records showed, however, that the Huddersfield sewage was frequently acid in reaction and despite liming the *p*H value was at times as low as 3·0 for several hours. This was due to discharge of acid from chemical works in the district. In view of Carpenter's (1928) generalization that the *p*H range of aquatic animals is approximately 4·7–8·5, it was anticipated that such a degree of acidity might be harmful to these oligochaets. The practice of dosing the Huddersfield beds with creosote in the spring to control the sewage fly *Psychoda alternata* Say. (Scouller & Goldthorpe, 1932) may also affect the worm population. However, before work on these two lines had progressed far, a routine check showed that the normal Huddersfield sewage was exerting a marked influence on the worm population independently of the *p*H, and the plan of experiments was altered accordingly.

The greater part of this paper deals with the toxicity of the Huddersfield sewage towards the eggs, young and adults of *Enchytraeus albidus* and *Lumbricillus lineatus*. In a third paper, these results will be related to those of the field work to explain the species composition, abundance and distribution of the worm population in the different Huddersfield bacteria beds. But the data are of general interest in revealing a differential reaction to toxic substances in closely related genera. They also have a practical bearing on the operation of the Huddersfield Sewage Plant.

Methods and scope of laboratory experiments

General. It was early apparent that some constituent of the Huddersfield sewage was markedly toxic to both species of worm. Field studies of the worm populations of four bacteria beds were proceeding parallel with the laboratory experiments, but owing to this toxic property the beds did not provide similar habitats. They can be divided into three types according to the sewage applied or the rate of application (see Fig. 1). Two of the beds were similar and formed part of the routine purification process, treating Huddersfield sewage at a rate of 100 gal./cu.yd./day; these beds are referred to as the Cooper Bridge beds (B3–4). The third and fourth beds

were used for experimental purposes by the Huddersfield authorities; one was treating sewage (the same as that applied to the Cooper Bridge beds) at a rate of 700 gal./cu.yd./day and it is called the Primary bed (B 1). The other, referred to as the Secondary bed (B 2), treated the sewage which had already passed through the Primary bed. Thus there were five sewages to be examined for toxicity, each differing in the degree of purification. The sewages applied to the Cooper Bridge and Primary beds although one and the same are always distinguished as Primary sewage (S 1) and Cooper Bridge sewage (S 3). The sewage leaving the Cooper Bridge beds is referred to as the Cooper Bridge effluent (E 3), that leaving the Primary bed as the Primary effluent (E 1). The sewage applied to the Secondary bed, to all intents and purposes the same as the Primary effluent, is referred to as the Secondary sewage (S 2), after treatment it is known as the Secondary effluent (E 2). More

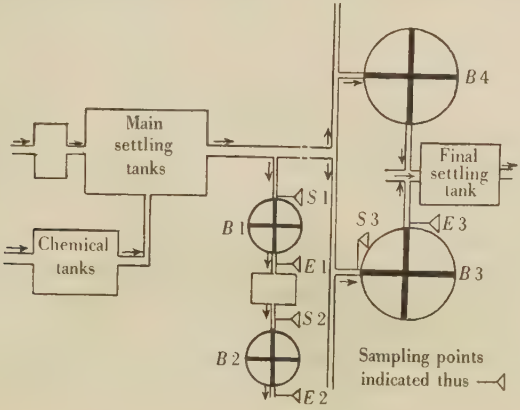

Fig. 1

detail of the Huddersfield sewage plant is given by Reynoldson (1947); the sewage sampling points are indicated in Fig. 1. The main purpose of the paper is to examine the toxicity of these five sewages and thereby provide data on the relative suitability of each bed for the two species of enchytraeid worm. Possible effects of acidity of the sewage were also studied.

Experiments on toxicity. The ability of adult worms, young worms and eggs of both species to tolerate the Huddersfield sewage was examined. In presenting the data each bed, B 1, B 2 and B 3–4, is treated separately in the first place. The toxicity of the sewage fed to a bed and that of the effluent leaving the bed was tested; intermediate stages of treatment could not be procured from within a bed. Samples of sewage were taken weekly and since the composition of sewage varies continuously and widely it was clearly impossible to cover all variations. But the general picture has emerged clearly enough. Experiments with each treatment stage were repeated four times using a different sample of sewage on each occasion.

The toxicity to adult worms of the sewage being fed to a bed and the toxicity of the same sewage (effluent) leaving the bed, was tested for both species at the same time. A control using Leeds bed effluent was also employed. Batches of ten worms of each species were immersed in a film of the fluid in separate watch glasses for a period of 7 days. The fluid was changed daily and the number of live worms at the end of the period recorded. A similar technique was employed for worms 3–8 days old (i.e. after hatching). For testing the toxicity of the Huddersfield sewage to eggs, cocoons were obtained by a method already described (Reynoldson, 1939*b*). Tubes were prepared in two series for each species. In the controls the pad of cotton wool containing the cocoons was moistened with Leeds bed effluent; in the other series, the pad was moistened with the particular Huddersfield sewage being tested. The fluid was changed daily until hatching occurred in the controls. Experiments were conducted between 14 and 16° C. and maintained approximately 10 days for *Lumbricillus lineatus* and 14 for *Enchytraeus albidus* (Reynoldson, 1943). Where a particular Huddersfield sewage had proved sufficiently toxic to kill the adults, the cocoons were obtained on pads moistened with Leeds sewage and transferred to Huddersfield sewage within 24 hr. of deposition. The room temperature during the experimental work was recorded and also the *p*H of the Huddersfield sewages. Further variations in procedure are described for particular experiments. Careful tests showed that Leeds effluent was harmless to all stages of the two species and could therefore be used as a control. All the *L. lineatus* were collected from the Leeds bacteria beds. However, the possibility of physiological adaptation in the Huddersfield *L. lineatus* was examined in so far as the material from these beds permitted. The effect of varying the temperature on the toxicity of Huddersfield sewage towards the eggs was examined at 7, 15 and 20° C. Preliminary study of the general properties of the toxic substance(s) was also commenced.

Results

Cooper Bridge beds (B 3–4)—the toxicity of sewage S 3 and effluent E 3

Sewages S 1 and S 3 were very similar and since the experiments were repeated independently for each bed the results of eight tests are available. Results from effluents E 1 and E 3 cannot be bracketed since the beds, B 1 and B 3–4, are operated at very different rates with a corresponding difference in the degree of oxidation of the sewage.

Adult worms. Table 1 summarizes the results of the experiments for both species; the *p*H records refer to S 3 only but the value always tends to neutrality as the sewage passes down the bed. The *p*H of the Leeds bed effluent was 6·5–7·5. The results show that *E. albidus* and *L. lineatus* differ in ability to tolerate S 3. It did not prove toxic to *E. albidus* whereas in two experiments (Exps. 2 and 3) most of the ten *L. lineatus* succumbed and all told twenty-nine (36%) died. The variation in composition of the Huddersfield sewage is also well illustrated, since in Exps. 5 and 8 it proved entirely harmless to *L. lineatus*. The ability of *L. lineatus*

to withstand effluent E3 suggests that the toxic substance(s) is broken down during passage through the bed. This point is emphasized by Exps. 2 and 3 where the sewage produced high mortality but the corresponding effluent was non-toxic. The death of five specimens of *E. albidus* in effluent E3 in Exp. 1 was regarded as accidental since the corresponding sewage was innocuous.

TABLE 1. *The toxicity of the Cooper Bridge sewage S3 and effluent E3 to adult* Lumbricillus lineatus *and* Enchytraeus albidus; *ten worms of each species were used in each experiment*

			No. dead					
			L. lineatus			*E. albidus*		
Exp.	*p*H	Temp. (° C.)	S3	E3	Control	S3	E3	Control
1	6·5	15·3	2	0	0	0	5	0
2	6·3	15·3	9 (9)	0	0	0	0	0
3	5·0	14·0	8	0	0	0	0	0
4	7·2	—	2	0	0	0	0	0
5	7·8	15·7	0	—	—	0	—	0
6	6·5	15·6	2	—	0	0	—	0
7	6·7	14·8	6 (6)	—	0	0	—	1
8	6·0	15·8	0	—	0	0	—	0
	Total	No. dead	29 (36 %)	0	0	0	5 (12 %)	1 (1 %)
		No. alive	51 (64 %)	40 (100 %)	70 (100 %)	80 (100 %)	35 (88 %)	79 (99 %)

TABLE 2. *The toxicity of Cooper Bridge sewage S3 and effluent E3 to young* Lumbricillus lineatus *and* Enchytraeus albidus; *ten worms of each species were used in each experiment*

			No. dead					
			L. lineatus			*E. albidus*		
Exp.	*p*H	Temp. (° C.)	S3	E3	Control	S3	E3	Control
1	6·5	15·3 (*L. l.*) 16·3 (*E. a.*)	10	0	0*	0	0	0
2	5·0	14·0	10	0	1	6	0	0
3	7·5	14·9	10	9	1	3	0	0
4	6·6	15·3	10	0	0	3	1	0
5	7·8	15·5	10 (10)	—	0	0	—	0
6	6·6	15·7	0 (0)	—	0	0	—	0*
7	6·6	14·9	4	—	0	3	—	0
8	—	14·6	3	—	0	0	—	6
	Total	No. dead	57 (71 %)	9 (22 %)	2 (3 %)	15 (19 %)	1 (3 %)	6 (9 %)
		No. alive	23 (29 %)	31 (78 %)	76 (97 %)	65 (81 %)	39 (97 %)	72 (91 %)

* Eight worms observed.

Young worms. The experimental conditions described for the adults also apply to the young worms. The results (Table 2) show that *E. albidus* was better able to withstand the sewage, suffering 19 % mortality compared with 71 % for *L. lineatus*. In both species, however, the young worms were more susceptible than the adults.

In *L. lineatus* there is an increase of 35% deaths and with *E. albidus* an increase of 19% when young and adults are compared. The same degree of variation in reaction to the different sewage samples is shown. Except in Exp. 3, effluent E3 proved non-toxic to both species. It is considered that the lethal property of this sample was exceptional, particularly as the lesser oxidized E1 proved non-toxic to young *L. lineatus* (Table 6).

Eggs. Owing to the labour involved in preparing material, it was impracticable to run concurrently experiments on both species with sewage S3, effluent E3 and a control. Emphasis was placed on a comparison of the two species and experiments performed concurrently with both using the effluent first. The eggs were contained

TABLE 3. *The toxicity of Cooper Bridge sewage S3 and effluent E3 to the eggs of* Lumbricillus lineatus *and* Enchytraeus albidus

Exp.	pH	Temp. (° C.)		*L. lineatus* S3	*L. lineatus* Control	*E. albidus* S3	*E. albidus* Control
1	6·3	14·4	No. observed	53	46	95	180
			No. developing	0	36	0	163
2	7·2	13·9	No. observed	—	—	77	91
			No. developing	—	—	10	78
3	7·4	14·7	No. observed	—	—	65	72
			No. developing	—	—	12	55
4	6·5	14·6	No. observed	—	—	42	64
			No. developing	—	—	0	43
	Total		No. observed	53	46	279	407
			No. developing	0	36	22 (8%)	339 (83%)
				E3	Control	E3	Control
1	6·6	14·2	No. observed	73	59	143	143
			No. developing	0	47	130	128
2	6·8	14·8	No. observed	104	119	152	143
			No. developing	0	83	143	128
3	7·0	14·0	No. observed	54	—	55	—
			No. developing	0	—	48	—
	Total		No. observed	231	178	350	286
			No. developing	0	130 (73%)	321 (92%)	256 (90%)

in the cocoons during the experiments as in nature. Since the effluent was tested first and proved highly toxic, totally preventing the development of the eggs of *L. lineatus*, there seemed no point in testing the more toxic sewage against these eggs. This accounts for the single experiment with the latter fluid, recorded in Table 3. In this one experiment Leeds sewage as opposed to effluent was used as a control since it had not been previously shown that Leeds sewage permits development. The result confirms that it does so, as the large worm population of these beds would suggest (Reynoldson, 1939*a*).

The eggs of both species are highly affected by S3; only 8% of the eggs deposited by *E. albidus* were able to develop. The environmental resistance offered to both species must therefore be extremely high in the surface layers of bed media. A striking contrast is shown by the reaction to E3 (Table 3). Again the difference

between the two species is marked. As already stated, none of the eggs of *L. lineatus* developed but those of *E. albidus* were unaffected. It should be noted that E3 is as favourable to the progress of the latter species as the Leeds effluent.

Normally, the Huddersfield sewage is brown in contrast to the grey cloudy appearance of domestic sewage. Frequently, the originally colourless cocoon wall of *L. lineatus* was stained brown by the Huddersfield sewage. Many of the eggs also became similarly stained while others remained white. This differential staining may have indicated a difference in reaction—development may have been only temporarily suspended in the eggs which resisted the stain. To test this, cocoons containing eggs of *L. lineatus* which had remained unstained were transferred to wool pads moistened with Leeds effluent. Fifty-seven eggs in the early stages of segmentation were observed but no further development occurred, the inhibition appearing to be permanent. Similar observations were made on *E. albidus* with the same general results. But here the eggs in one cocoon which had failed to develop in S3 sewage did so when transferred to Leeds effluent, suggesting that intermittent progress could occur in this species as the sewage varied in composition.

TABLE 4. *The effect of Huddersfield sewage on egg production in* Lumbricillus lineatus *and* Enchytraeus albidus

	L. lineatus			*E. albidus*		
Sewage	Worms used	Eggs produced	Eggs per worm	Worms used	Eggs produced	Eggs per worm
Leeds bed effluent	128	602	4·7	30	186	6·2
Sewage S3	30	53	1·8	59	172	3·0
Effluent E3	60	177	3·0	77	386	5·0

Two additional experiments were performed with the eggs of *E. albidus*, using in the first an S3 sewage which may have contained less chemical wastes and was not the usual brown colour; in the second, a sewage diluted by a rapid snow thaw. Twenty-eight and seventy-eight eggs respectively were observed in each test and 86 and 32% developed normally. Therefore periods of lowered antagonism did occur intermittently and dilution by rainwater may be of importance in permitting a higher rate of population increase from time to time.

Egg production. A record of the number of worms depositing the eggs used in the foregoing experiments provided data on the effect of sewage S3 and effluent E3 on the rate of egg production. The results are presented in Table 4; the figures of egg production in Leeds bed effluent are taken from Reynoldson (1943). S3 influences both species; over a 5-day period the egg production was reduced by 62% with *L. lineatus* and by 52% for *E. albidus*. E3 had a smaller effect and the corresponding figures were 36 and 19% respectively; it is doubtful if the latter result represents a real difference. In all experiments the incubation periods of the eggs, irrespective of the sewage used, were not significantly different from those observed in Leeds

effluent, at the equivalent temperatures; i.e. toxicity did not influence developmental rate.

To summarize; these experiments show that there is a marked difference in the ability of *L. lineatus* and *E. albidus* to tolerate the Huddersfield sewage. For both species the young worms were less tolerant than adults and eggs still less. Toxicity of the sewage was reduced by passage through the bed, probably as the result of oxidation. The sewage was particularly deleterious to *L. lineatus* since about half of the adults and more than half of the young worms succumbed, while egg development was totally prevented. The effluent E3 was less drastic, both young and adults survived but the eggs were still unable to make progress. The harmful effects on *E. albidus* were less marked and only the eggs were adversely influenced by the sewage; the effluent E3 was non-toxic.

Primary bed (*B* 1)—*the toxicity of the Primary effluent* (*E* 1)

The toxicity of the Primary sewage S1 was (p. 333) similar to that of S3; it remained therefore to examine the toxicity of effluent E1. In actual operation

Table 5. *The toxicity of the Primary effluent E* 1 *to adult* Lumbricillus lineatus *and* Enchytraeus albidus; *ten worms of each species were used in each experiment*

			No. dead			
			L. lineatus		*E. albidus*	
Exp.	*p*H	Temp. (° C.)	E1	Control	E1	Control
1	6·7	15·7	1	—	0	0
2	6·6	15·6	0	0	1	0
3	6·9	14·8	1 (0)	0	0	1
4	6·0	15·8	0	0	0	0
	Total	No. dead	2 (5%)	0	1 (2%)	1 (2%)
		No. alive	38 (95%)	30 (100%)	39 (98%)	39 (98%)

the main difference between beds B1 and B3-4 lay in the rates at which sewage was applied: during the period of study these averaged 700 and 100 gal./cu.yd. of filtering media per day, respectively. The overall oxidation of the sewage based on the Biochemical Oxygen Demand test (see Ministry of Health publication, *Methods of Chemical Analysis as applied to Sewage and Sewage Effluents*), was approximately 50% for B1 and 75% for B3–4 (Goldthorpe & Nixon, 1942). If, as the results in the preceding section suggest, toxicity is reduced by oxidation then the effluent from B1 should be more harmful than that from B3–4. The experiments were made using the same techniques as employed for effluent E3.

Adult worms. The results of these experiments are recorded in Table 5; only two (5%) of the forty *L. lineatus* succumbed compared with 36% in the sewage (Table 1). Thus, the toxic property of S1 towards adult worms had been almost completely removed. Comparison of Exp. 7 in Table 1 with Exp. 3 in Table 5 for which the same Huddersfield sewage, before and after treatment in bed B1, was used, affords

more precise information on this point. Whereas in the sewage six worms out of ten died, in the effluent only one succumbed. The results with *E. albidus* were confirmatory since it had already been shown that the adults could live in sewage S3. The young worms of both species were also able to tolerate effluent E1 (Table 6). Exp. 4 in this table, recording the failure of six *E. albidus* to survive, is regarded as unrepresentative—this stage seemed particularly sensitive to handling and occasionally deaths were recorded from control experiments.

TABLE 6. *The toxicity of the Primary effluent E1 to young* Lumbricillus lineatus *and* Enchytraeus albidus; *ten worms of each species were used in each experiment*

			No. dead			
			L. lineatus		*E. albidus*	
Exp.	*p*H	Temp. (° C.)	E1	Control	E1	Control
1	6·7	15·5	0 (0)	0	0	0
2	6·3	15·7	0 (0)	0	0*	—
3	6·9	14·9	0	0	0	—
4	—	14·6	0	6	6	—
	Total	No. dead	0	6 (15%)	6 (12%)	0
		No. alive	40 (100%)	34 (85%)	32 (84%)	10 (100%)

* Eight worms observed.

TABLE 7. *The toxicity of the Primary effluent E1 to the eggs of* Lumbricillus lineatus *and* Enchytraeus albidus

			L. lineatus	
Exp.	*p*H	Temp. (° C.)	No. observed	No. developing
1	7·4	14·8	73	0
2	7·3	14·0	70	0
3	7·1	15·0	49	0
Total			192	0
			E. albidus	
			No. observed	No. developing
1	7·2	14·8	65	31 (48%)
2	7·4	14·7	72	53 (74%)
3	6·9	14·7	64	43 (67%)
4	6·9	14·8	52	48 (92%)
Total			253	177 (70%)

Eggs. As expected, eggs of *L. lineatus*, which were inhibited by E3, were unable to develop in E1 (Table 7). There was insufficient material to provide a control for *E. albidus* but earlier experiments had established that 80–90% of the eggs deposited were fertile. The inhibitory effect of E1 clearly lay between those of S3 and E3. The percentage of eggs developing in the three treatment stages were: S3, 8%; E1, 70%; and E3, 92%.

The figure for E 1 would have been lower if Exp. 4 had not shown such a high proportion of eggs developing. However, there is no additional evidence that this particular sewage was less harmful than the average. Two further experiments at 7° C. gave the following results:

	Total eggs	Eggs developing
Exp. 1	78	51 (65%)
Exp. 2	61	42 (69%)

These additional data support the conclusion that E 1 inhibits development, but the effects may be exaggerated by the low temperature (p. 342).

Since both the beds B 1 and B 3–4 treat the same sewage, a measure of the relative antagonism of these two environments is given by comparing the toxic properties of the two effluents. Both beds should be highly unsuitable for *L. lineatus*. With *E. albidus*, B 3–4 might be expected to show the higher population because the effluent E 3 was innocuous to all stages whereas that from B 1 was harmful to the eggs.

Secondary bed (B 2)—the toxicity of the Secondary effluent (E 2)

It can be assumed that the degree of toxicity of the Secondary sewage S 2 was very close to that of effluent E 1 (p. 333); therefore we are concerned here only with

TABLE 8. *A summary of the toxicity of the sewages based on percentage mortality, arranged to indicate the abiotic resistances of the three bed types. The mean values for B 1 and B 2 are calculated from S 3–E 1 and E 1–E 2 respectively*

	Primary bed (B 1)				Cooper Bridge beds (B 3–4)						Secondary bed (B 2)			
	E. albidus		*L. lineatus*		*E. albidus*			*L. lineatus*			*E. albidus*		*L. lineatus*	
	E 1	Mean	E 1	Mean	S 3	E 3	Mean	S 3	E 3	Mean	E 2	Mean	E 2	Mean
Adult	2	1	5	20·5	0	12	6	36	0	18	0	1	0	2·5
Young	12	15·5	0	35·5	19	3	11	71	22	46·5	0	6	0	0
Eggs	30	61	100	100	92	8	50	100	100	100	14	22	100	100

effluent E 2. Furthermore, at this stage of treatment the sewage is non-toxic to adults and young of both species (Tables 5, 6) and attention could be confined to the eggs. Bed B 2 was treating sewage already partly oxidized in B 1, at 279 gal./cu.yd./day and this bed was expected to be the most favourable for both species. During the period October 1939 to October 1940 80% oxidation of sewage S 3 was recorded after passage through B 2. This compares with 75% for E 3, and since the latter failed to inhibit development of the eggs of *E. albidus* it might be assumed that E 2 would likewise prove harmless. Two independent checks involving observation of forty-four and twenty-nine eggs showed that 93 and 76% respectively developed normally, confirming that this effluent was non-toxic.

The main purpose of these experiments was to determine if sufficient of the toxic material(s) had been removed by passage through B 2 to permit development of

L. lineatus eggs. In three tests, sixty-one, fifty-eight and seventy-seven eggs were observed and, in all, development failed to occur.

The relative suitability of each type of bed, B 1, B 2 and B 3–4, as determined by the sewage, is indicated in Table 8 which summarizes the data of Tables 1–3 and 5–7. Clearly, B 1 was most antagonistic to the worms, B 2 the least while B 3–4 lay between. These results are related to the worm population of each bed in the third paper of the series.

Following the decline of the worm population in the spring of 1940 (Reynoldson, 1947), certain of the experiments previously described were repeated in July and August 1940 to determine whether toxicity had increased. The results were essentially similar to those already recorded and it must be concluded that the drop in worm numbers was not related to any change in composition of the sewage.

Physiological adaptation in Lumbricillus lineatus

The bulk of the experimental data on *L. lineatus* refers to worms obtained from the Leeds bacteria beds. Evidence suggesting that strains of organisms resistant to toxic substances can be produced by selection in a relatively short time (Dobzhansky, 1937) instils caution in applying the experimental data. The supply of *L. lineatus* from the Huddersfield beds sufficed to make only isolated tests for such physiological adaptation. The results obtained with these adult and young worms are recorded in parentheses alongside those for Leeds *L. lineatus* in Tables 1, 2, 5 and 6. The reaction of both strains of this species was very similar and these data gave no indication of any adaptation. This was supported by the results obtained with eggs deposited by Huddersfield *L. lineatus*: sixty-six were tested against effluent E 3 and none showed any signs of development beyond the initial segmentation stages. A similar result was obtained with sixteen such eggs tested against E 2. If physiological adaptation had occurred it might be expected to reveal itself in the latter experiment in which the least toxic sewage was used.

Temperature and toxicity

All experiments to examine the toxic effect of the Huddersfield sewage upon the eggs were performed at approximately 15° C. However, breeding being most active during the colder months when the range within the bed is 9–12° C., it follows that such information, to be most useful, must take into account the possible conditioning influence of temperature. Experiments were made at 7, 15 and 20° C., covering the annual temperature range experienced in the beds at the 12-in. level, a range much damped compared with that of the air temperature (Lloyd, 1935). Clear-cut results were obtained with *L. lineatus* using effluent E 2, the least toxic material, the reaction of the eggs at these three temperatures being precisely similar. The experiment was repeated three times at each temperature and not one of the 400 eggs observed showed any advanced segmentation. A rather different result

(Table 9) was obtained with the eggs of *Enchytraeus albidus* in S 3. Here the proportion of eggs developing normally increased as the temperature was raised; for example, only 5% of the eggs developed at 7° C. but 48% at 20° C. A repetition at 15 and 20° C. showed a similar influence. Reynoldson (1943) demonstrated that the range of 7–20° C. did not affect egg fertility, therefore such a result was not produced by temperature *per se*. It would arise if only the early developmental stages were sensitive to the toxic substances in the sewage. The more rapid development at the higher temperatures will reduce the extent of the sensitive period and the cocoon also may be expected to exert some initial protection. The precise developmental stage at which resistance became sufficiently high to permit full development could only be determined microscopically and this lay outside the scope of the present work. Low temperature did not in any way reduce the viability of the eggs of *E. albidus* in E 3. A progressive falling off in sensitivity to Huddersfield sewage occurs through the entire life cycle from egg to adult and these experiments indicated that the same tendency was present from the early segmentation stages. Thus in

Table 9. *The effect of temperature upon the toxicity of the Huddersfield sewage towards the eggs of* Enchytraeus albidus

Exp.	Sewage used	Temp. (° C.)	Eggs observed	% eggs developing	Incubation period (days)
1	S 3	7	64	5	33
	S 3	15	65	18·5	14
	S 3	20	62	48	10
2	S 3	15	59	27	—
	S 3	20	59	80	—

the Huddersfield bacteria beds *E. albidus* may suffer a disadvantage in breeding during the winter months (Reynoldson, 1947); a much reduced egg mortality would be incurred in the upper layers of clinker during the summer.

A preliminary study of the toxic substances

There are two main contributors of chemical wastes, Messrs L. B. Holliday's Chemical Works and Imperial Chemical Industries (Reynoldson, 1947). The toxicity might arise from either of these wastes or from the domestic portion. Data on this subject might prove valuable to the Sewage Works Manager in planning modifications to the treatment plant. In all the present experiments the susceptible *L. lineatus* eggs were used as the 'test organism'. Each of the three component wastes was examined separately, and also the mixture of the constituents in the proportion in which they normally occur in the Huddersfield sewage. In the domestic sewage ninety-six (79%) out of 122 eggs developed to the hatching stage so that no inhibitory substance was present in this particular sample. Two hundred and sixty eggs equally apportioned among the three remaining sewages were observed, but none developed. Similar results were obtained when Messrs L. B. Holliday's waste

was diluted with domestic sewage in the proportion which normally occurs; fifty-three and fifty-one eggs were used in two tests of this kind. A second sample of domestic sewage was tested and proved harmful; only twelve out of thirty-four eggs developed and six of these showed pronounced abnormalities. Despite this it is probable that the domestic portion of the sewage is usually non-toxic to all stages of both species of worm. It seems clear that both Messrs L. B. Holliday's and Imperial Chemical Industries' discharges are toxic to the eggs of *L. lineatus*.

The toxicity of distillate and residue was examined by distilling L. B. Holliday's waste diluted with domestic sewage at a *p*H of 7·0. The results of two experiments agreed in showing that the residue still totally inhibited the development of the eggs of *L. lineatus*; all the 130 eggs employed failed to make progress. The distillate revealed a lowered toxicity and in one test, out of thirty-two eggs two developed normally, eighteen showed abnormal development and the remaining twelve aborted; in the second experiment the corresponding figures were sixteen, eighteen and seventeen respectively. The abnormal worms showed increased girth, malformation of the alimentary canal and blister-like swellings on the epidermis.

The results in Table 8 suggest that toxicity was reduced as a result of bacterial oxidation while the sewage percolated through the bed. If this was so it should be possible to remove the toxicity by prolonging oxidation. Accordingly, one of the samples of effluent E3 was kept in a bottle at 14° C. and tested at intervals. After 20 days the effluent still totally inhibited development of the eggs of *L. lineatus* (sixty-seven observed). But after 25 days a further test showed seventy-nine out of ninety-nine eggs developing. Of this number, twenty-four reached the stage of elongation only, forty developed into abnormal worms and failed to hatch while fifteen developed normally and hatched. In another sample of E3 the innocuous stage was reached after 28 days while in a third the toxic property was lost after 7 weeks. As the last-named was not tested at intervals the actual time taken for the breakdown of the poisonous substance(s) was not known. Such a result strengthens the suggestion that oxidation is involved. It is of practical interest to record that the toxic property could be removed by standing the effluent over activated charcoal for 12 hr. This was confirmed in three tests using 266 eggs. Such treatment also removed the colouring matter in the Huddersfield sewage. It is possible that some such treatment, although very costly, would permit colonization of B3 and B4 by a wider range of macro-organisms.

The effects of the acid sewage on the worms

Toxicity of the sewage was persistent and to gauge the relative importance of the effects of acidity it is necessary to consider degree and frequency. For this the charts recording the *p*H of sewage S3 were examined. Over 3 months, a *p*H of approximately 3·0 was recorded on five occasions, persisting $\frac{1}{2}$–$5\frac{1}{2}$ hr.; a *p*H of 3·5 was recorded fourteen times persisting 1–10 hr. (average $3\frac{1}{4}$ hr.) and a *p*H of 4·0 on nineteen occasions, over 2–$11\frac{1}{2}$ hr. (average $5\frac{1}{4}$ hr.). Laboratory experiments to test

the viability of both species in Huddersfield sewage acidified with HCl and H_2SO_4 showed that they reacted similarly in either medium. Adult worms died after 50–75 min. in a *p*H of 3·5 and after 4½ hr. in a *p*H of 4·0 but were able to survive up to 24 hr. when the *p*H was 4·5. Young worms were less tolerant, succumbing after 18 min. at *p*H 3·5 and after 2 hr. at *p*H 4·0. The effect on the eggs of *Enchytraeus albidus* was not examined but any effect on the eggs of *Lumbricillus lineatus* could not be important. Evans (unpublished) has shown that the mucus secreted under acid conditions has a very marked buffering effect. But as such protective secretions would be continually removed in the beds it may be that field conditions were more severe than these experiments suggest. On the other hand, data supplied by the Huddersfield Corporation indicated that the maximum harmful effect was confined to the upper part of the bed. The following records of the *p*H of sewage S 1 and that of the corresponding effluent E 1, show how rapidly neutralization is achieved:

Test	*p*H of S 1	*p*H of E 1
(1)	3·7	6·0
(2)	4·5	6·1
(3)	2·8	6·1

Neutralization would be even more rapid in beds B 3 and B 4 with a slower feed. For this reason, as well as the intermittent occurrence, the effect of acidity on the distribution of *E. albidus* in these beds is considered of secondary importance; under more normal conditions the effects of such concentrated acidity on a worm population would be detectable. Acid conditions could not react on the population of bed B 2 because here the acid was neutralized by passage through B 1 and the *p*H never fell below 5·9. But B 2 could not be used as a control in assessing the influence of *p*H on its own account owing to the interfering effect of toxicity on the population of B 3 and B 4.

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THE PRACTICAL CONTROL OF WIREWORM BY γ-BENZENE HEXACHLORIDE ('GAMMEXANE'): COMPARISONS WITH DICHLORODIPHENYLTRICHLORETHANE (D.D.T.)

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Benzene hexachloride containing 13% of the insecticidally active gamma isomer known as 'Gammexane' has been used successfully in a considerable number of field trials in the control of wireworm. On heavily infested land the effective dosage rates associated with an appreciable reduction in wireworm population, and giving a marked improvement in plant establishment and substantial increases in yield, range between 1 and 6 lb./acre (2–12 oz./acre of Gammexane), on oats and wheat. The effective dosage rates vary and depend upon whether the insecticide is combine-drilled, broadcast, or applied as a seed dressing.

Although D.D.T. applied by similar methods was associated with similar reductions in wireworm population, the use of this insecticide resulted in smaller yield increases.

Introduction

The control of wireworms has been a subject of study since the earliest days of applied entomology and it has presented the entomologist with a problem apparently incapable, in a number of instances, of practical solution. Present control measures are often uneconomic or are either unreliable or inadequate. They include the use of soil insecticides or fumigants, seed protection, repellents, and certain cultural practices which have become conventional under English conditions.

The outbreak of war in 1939 and the subsequent national campaign to bring additional permanent pastures under the plough led to increased efforts to combat this pest. Wireworm surveys of grassland under Government auspices were organized and they proceeded as a measure of high priority. They had as their object the provision of information on wireworm population in ploughed up grassland so that resistant crops could be planted under conditions where cereals were likely to fail.

The details of these surveys have been published by the Ministry of Agriculture (Anon., 1944) and the broad conclusions are that fields with wireworm populations of 300,000 per acre or under are safe for the cultivation of all farm crops except potatoes, and perhaps sugar beet and mangolds. Populations between 300,000 and 600,000 per acre are considered safe for cereals. Populations greater than 600,000 per acre are said to be risky on light land for cereal crops but are less so in heavier soils, whilst populations over 1,000,000 per acre are potentially dangerous to most farm crops.

This information was valuable since it made possible the selection of resistant crops for planting in those soils with known high wireworm populations. This

procedure, however, necessarily limited the choice of crops, and an effective direct control measure which would avoid this was acknowledged to be clearly advantageous.

Between 1939 and 1941, and concurrent with these surveys, considerable work was carried out by Hawthorndale Laboratories, Jealott's Hill Research Station, Berks, to find efficient chemical means of controlling wireworm. None of the results obtained during this period justified further development.

Further work in these laboratories during 1942 and 1943 led to the discovery of the insecticidal properties of the gamma isomer of benzene hexachloride referred to as Gammexane by Slade (1945).

This discovery opened up new possibilities and experiments with the insecticide on wireworms were initiated in 1943.

Experimental

The material used in the preliminary work was crude benzene hexachloride (B.H.C.) as manufactured by Imperial Chemical Industries, General Chemicals Division. It consisted of a mixture of the various isomers (α, β, γ, δ, etc.) and had a gamma isomer (Gammexane) content of 13%.

After laboratory and small-scale trials had established dosage rates that would not be harmful to cereals, a trial was laid down on Jealott's Hill Farm in the autumn of 1943 to check these findings on a practical scale. The wartime priority of the problem and the need to consider immediate practical aspects led to the first trial being laid down when a minimum of data on toxicity of the insecticide to wireworm was available.

The Drownboy field experiment

The first full-scale insecticidal trial with benzene hexachloride was laid down in the spring of 1944 in Drownboy field, Jealott's Hill Research Station. This field was selected for experiments after wireworm sampling, the experimental area being divided into randomized replicated strips. Each strip extended the length of the field and was the width of four standard drills, i.e. some 30 ft. There was one treatment and one untreated control replicated four times. The treatment consisted of $\frac{1}{2}$ cwt./acre of 2·5% benzene hexachloride combine-drilled with oats.

In the more heavily infested part of the field a transverse section across the strips was taken to provide $\frac{1}{20}$-acre plots. Wireworm populations were determined in these areas after harvest by taking eight random soil samples (4 in. cores) per plot—four on and four between the drill rows. Plant populations were determined by taking at random the numbers of seedlings before tillering in twenty 1-yd. lengths of drill row. Yields were determined by harvesting the whole of the $\frac{1}{20}$-acre plots. Details of the effects of the treatment on wireworms and plant populations, together with yield data, are given in Table 1.

The benzene hexachloride dust used in this trial flowed freely through the combine-drill at a rate estimated at approximately 56 lb./acre. No effects were noticed until 5 weeks after sowing when gaps began to appear in the untreated

control plots. Braird counts taken before tillering and 5½ weeks after sowing, showed that the plant population of treated plots was nearly double that of the untreated plots. Yield data obtained revealed that the weight of grain had also been doubled, increasing from 6·5 cwt./acre on untreated control to some 13 cwt./acre on the treated plots. This yield increase, accompanied by a substantial reduction in the wireworm population as recorded, provided the first positive evidence of the effectiveness of benzene hexachloride.

TABLE 1. *Effect of combine drilled benzene hexachloride on wireworms and plant populations and yield of oats in Drownboy field*

Treatment	Plants/1 yd. drill row	Wireworms (1000's/acre)	Yield (cwt./acre)	
			Straw	Grain
		Actual data		
2·5% crude benzene hexachloride (½ cwt./acre)	41	75	21·6	13·0
Untreated	23	225	13·0	6·5
		Data calculated on basis of untreated = 100		
2·5% crude benzene hexachloride (½ cwt./acre)	178	33·3	166	200
Untreated	100	100	100	100

Extension trials

The striking results obtained with combine-drilled benzene hexachloride in the Drownboy trial led to the planning of extension field trials which were laid down mostly in the neighbourhood of Jealott's Hill, Berks, with some in Bucks and Essex, during 1944, 1945 and 1946. The object of these trials was to investigate dosage rates and also to assess the practicability and efficiency of applying this insecticide on a field scale by other methods. The trials were designed to give results of immediate practical value rather than fundamental toxicity data. As the efficiency of dichlorodiphenyltrichlorethane against wireworm had not previously been determined at Hawthorndale, this chemical in the form of its technical grade referred to in the text as D.D.T. and containing 70–75% 1:1-*bis*-(*p*-chlorophenyl)-2:2:2-trichlorethane, was included in certain of the trials. The benzene hexachloride insecticides were applied by broadcasting, combine drilling, and, because of the small amounts of Gammexane which seemed to be required as indicated by the Drownboy trial, as seed dressings. The D.D.T. preparations were applied by combine drilling and as seed dressings.

The information on critical levels of wireworm population given in the Ministry survey provided a basis for the selection of suitable fields. Those chosen, mostly first year after grass, had populations per acre ranging from 500,000 to 1,500,000 wireworms of over 5 mm. in length.

Nineteen trials were laid down mainly on winter wheat in the autumn of 1944, and thirty-three, mainly oats, in the spring of 1945. These trials, like the Drownboy

field trial, consisted of suitably replicated strips of 2–4 drill widths traversing the field. Of these fifty-two trials, nineteen, where wireworm attack was severe, were selected for completion. The data were in general obtained from plots, 30 yd. in length, formed by a selected section traversing the strips at right angles. Counts on these plots were taken to determine plant and wireworm populations. Plant populations were determined in each plot by taking, at random, the numbers of seedlings before tillering in twenty to fifty 1-yd. lengths of drill row. Wireworm populations were determined by taking eight random soil cores, (4 in. diam. × 6 in. deep) per plot—four samples on and four samples between the drill rows. Yields of straw and grain were determined by taking, in each plot, the produce from ten areas, each of 1 sq.yd., at regular intervals along the length of the plot.

(*a*) *Application by combine drilling*

(1) *Wheat.* Of the nineteen trials originally laid down, mainly on autumn sown wheat, four were selected for completion. In these trials the following dusts were compared:

(i) 2% benzene hexachloride on a gypsum base;
(ii) 2% benzene hexachloride on a superphosphate base;
(iii) 2% D.D.T. on a gypsum base.

All were applied at 1 cwt./acre. The summarized results are given in Table 2.

TABLE 2. *Effects on yields, in cwt./acre, of benzene hexachloride and D.D.T. dusts, combine drilled on autumn wheat*

Trial	2% benzene hexachloride on gypsum at 1 cwt./acre		2% benzene hexachloride on superphosphate at 1 cwt./acre		2% D.D.T. on gypsum at 1 cwt./acre		Untreated		Significant difference: Straw		Significant difference: Grain	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	0·05	0·01	0·05	0·01
	Actual data											
No. 2, Littlewick Green, Berks	42·0	28·4	48·2	30·0	25·8	15·2	18·4	12·1	10·63	14·1	6·36	8·44
No. 3, Olney, Bucks	—	—	42·6	24·6	—	—	16·7	10·5	4·64	6·15	2·68	3·55
No. 4, Essex A	47·3	28·6	57·4	32·6	49·0	25·8	28·7	14·7	8·27	11·09	5·21	6·99
No. 5, Essex B	94·5	39·5	79·0	37·8	70·2	33·6	49·4	24·7	11·49	15·40	5·10	6·82
Means of nos. 2, 4 and 5*	61·3	32·2	61·5	33·5	48·3	24·9	32·2	17·2	6·13	8·08	3·40	4·50
	Data calculated on basis of untreated = 100											
No. 2, Littlewick Green, Berks	228	235	262	248	141	126	100	100	58	77	53	70
No. 3, Olney, Bucks	—	—	255	243	—	—	100	100	28	37	26	34
No. 4, Essex A	165	195	200	222	171	176	100	100	29	39	35	48
No. 5, Essex B	191	160	160	153	142	136	100	100	23	31	21	28
Means of nos. 2, 4 and 5*	191	187	191	195	150	145	100	100	19·1	25·0	19·8	26·2

* Data from no. 3, which is incomplete, have not been included in calculating means.

In this series of trials it was possible to estimate wireworm populations only in trial no. 2, Littlewick Green, Berks. These, in 1000 per acre, were as follows:

2% benzene hexachloride on gypsum at 1 cwt./acre	2% benzene hexachloride on superphosphate at 1 cwt./acre	2% D.D.T. on gypsum at 1 cwt./acre	Untreated
440	375	470	1030

It can be seen that, at 2 lb./acre, benzene hexachloride and D.D.T. reduced the wireworm population by more than 50%. The former increased the yield to approximately two and a half times and the latter to one and a quarter times that of the untreated control. In the remaining three trials, where data on wireworm populations are not available, yields were double and one and a half times that of untreated in the benzene hexachloride and D.D.T. plots respectively.

TABLE 3. *Effects of benzene hexachloride and D.D.T. on wireworm and plant populations and on yield when combine drilled with spring oats*

		2 % benzene hexachloride dust at ¾–1 cwt./acre	4 % benzene hexachloride granules at ½ cwt./acre: Without C.C.F.*	4 % benzene hexachloride granules at ½ cwt./acre: With 1½–2½ cwt./acre of C.C.F.*	2 % D.D.T. dust at ¾ cwt./acre	Untreated	Significant difference 0·05	Significant difference 0·01
				Actual data				
Trial 7, Wokingham, Berks	Wireworms 1000's/acre	690	625	1020	875	1420	575	760
	Plants/1 yd. drill row	20	18	24	14	11	8·78	11·89
	Yield cwt./acre Straw	26·2	28·8	34·0	16·7	12·5	5·20	6·86
	Yield cwt./acre Grain	15·3	15·1	20·6	9·1	6·7	3·10	4·09
Trial 8, Wokingham, Berks	Wireworms 1000's/acre	155	230	210	520	355	275	365
	Plants/1 yd. drill row	36	36	37	32	33	6·24	8·33
	Yield cwt./acre Straw	17·1	14·1	27·1	12·4	10·9	3·74	4·94
	Yield cwt./acre Grain	4·5	4·4	6·6	2·6	2·7	0·91	1·21
Trial 9, Warfield, Berks	Wireworms 1000's/acre	730	915	1125	875	1335	535	705
	Plants/1 yd. drill row	23	16	21	16	11	2·89	3·88
	Yield cwt./acre Straw	30·8	26·5	42·1	20·6	6·9	4·02	5·29
	Yield cwt./acre Grain	13·9	13·0	19·9	7·6	4·3	1·88	2·48
Means	Wireworms 1000's/acre	525	523	785	753	1037	275	365
	Plants/1 yd. drill row	26	23	27	21	18	3·47	4·60
	Yield cwt./acre Straw	24·7	23·1	34·4	16·6	10·1	2·61	3·44
	Yield cwt./acre Grain	11·2	10·8	15·7	6·4	4·6	1·33	1·76
				Data calculated on basis of untreated = 100				
Trial 7, Wokingham, Berks	Wireworms 1000's/acre	48·6	44	72	61·7	100	40·5	53·5
	Plants/1 yd. drill row	182	164	218	127	100	80	108
	Yield cwt./acre Straw	210	230	272	131	100	42	55
	Yield cwt./acre Grain	228	226	307	136	100	46	61
Trial 8, Wokingham, Berks	Wireworms 1000's/acre	43·7	64·8	59·2	146	100	77·4	103
	Plants/1 yd. drill row	109	109	112	97	100	19	25
	Yield cwt./acre Straw	157	129	249	114	100	34	44
	Yield cwt./acre Grain	167	163	245	96	100	35	45
Trial 9, Warfield, Berks	Wireworms 1000's/acre	55	68·5	84·5	65·5	100	40·0	52·8
	Plants/1 yd. drill row	209	145	191	145	100	26	35
	Yield cwt./acre Straw	446	384	610	298	100	58	77
	Yield cwt./acre Grain	323	302	464	177	100	44	58
Means	Wireworms 1000's/acre	50·6	50·4	76	73	100	26·6	35·2
	Plants/1 yd. drill row	144	128	150	117	100	19	26
	Yield cwt./acre Straw	245	229	340	163	100	26	34
	Yield cwt./acre Grain	243	235	342	139	100	29	38

* I.C.I. Concentrated Complete Fertilizer.

(2) *Oats.* Of the seven field trials laid down in the spring of 1945, four were completed. In three of these, benzene hexachloride in powder and granular forms—the latter with and without complete fertilizer—was compared with a powder preparation of D.D.T. All treatments were combine drilled with spring oats. The fourth trial, trial no. 6, Hurst, excluded D.D.T. and included nitro-chalk in some of the treatments and was therefore not comparable with others in this series.

In Table 3 it can be seen that in the two most heavily infested fields in the Wokingham and Warfield trials, benzene hexachloride in powder and granular forms applied at about 2 lb. benzene hexachloride per acre produced mean grain and

straw yields of about two and a half times those of untreated plots. The addition of about 1½ cwt./acre of granulated complete fertilizer (I.C.I. Special no. 2) to the granular benzene hexachloride preparation, more than trebled the yield. It is of incidental interest to note that reduction in the wireworm population was generally less where the complete fertilizer had been applied. It will be seen that D.D.T., compared with benzene hexachloride at comparable rates of application, i.e.

TABLE 4. *Comparison of broadcasting and combine drilling benzene hexachloride on spring oats*

		2 % B.H.C. dust broadcast in seed-bed and harrowed in at following rates per acre			2 % B.H.C. dust combine drilled with seed at following rates per acre		Untreated	Significant difference	
		1½ cwt.	3 cwt.	6½ cwt.	¾ cwt.	1–1½ cwt.		0·05	0·01
					Actual data				
Trial 10, Warfield, Berks	Wireworms 1000's/acre	—	280	315	—	375	1280	520	690
	Plants/1 yd. drill row	—	25	33	—	22	14	9·16	12·34
	Yield cwt./acre Straw	—	36·3	42·6	—	29·5	7·0	6·16	8·17
	Yield cwt./acre Grain	—	18·1	22·0	—	15·0	3·6	3·23	4·29
Trial 11,* Wokingham, Berks	Wireworms 1000's/acre	190	280	—	155	—	560	330	440
	Plants/1 yd. drill row	26	22	—	23	—	17	4·80	6·73
	Yield cwt./acre Straw	18·9	18·3	—	20·0	—	13·0	4·61	6·10
	Yield cwt./acre Grain	4·9	5·4	—	5·1	—	3·7	1·34	1·78
Trial 12, Warfield, Berks	Wireworms 1000's/acre	—	—	530	—	375	1000	555	740
	Plants/1 yd. drill row	—	—	31	—	25	17	6·75	9·35
	Yield cwt./acre Straw	—	—	35·0	—	29·6	18·6	5·31	7·07
	Yield cwt./acre Grain	—	—	20·4	—	15·6	9·4	2·10	2·79
Trial 13, Binfield, Berks	Wireworms 1000's/acre	435	—	—	—	375	1095	705	940
	Plants/1 yd. drill row	51	—	—	—	40	12	11·64	16·74
	Yield cwt./acre Straw	34·4	—	—	—	30·7	8·3	4·76	6·36
	Yield cwt./acre Grain	16·5	—	—	—	14·7	5·0	2·29	3·06
					Data calculated on basis of untreated = 100				
Trial 10, Warfield, Berks	Wireworms 1000's/acre	—	21·9	24·6	—	29·3	100	41	54
	Plants/1 yd. drill row	—	179	236	—	157	100	65	88
	Yield cwt./acre Straw	—	520	610	—	421	100	88	117
	Yield cwt./acre Grain	—	503	610	—	417	100	90	119
Trial 11,* Wokingham, Berks	Wireworms 1000's/acre	34	50	—	28	—	100	59	78
	Plants/1 yd. drill row	153	130	—	135	—	100	28	40
	Yield cwt./acre Straw	145	141	—	154	—	100	35	47
	Yield cwt./acre Grain	132	146	—	138	—	100	36	48
Trial 12, Warfield, Berks	Wireworms 1000's/acre	—	—	53	—	37·5	100	55·5	74·0
	Plants/1 yd. drill row	—	—	182	—	147	100	40	55
	Yield cwt./acre Straw	—	—	188	—	159	100	29	38
	Yield cwt./acre Grain	—	—	217	—	166	100	22	30
Trial 13, Binfield, Berks	Wireworms 1000's/acre	39·6	—	—	—	34·2	100	64·3	85·5
	Plants/1 yd. drill row	425	—	—	—	333	100	97	137
	Yield cwt./acre Straw	415	—	—	—	370	100	57	77
	Yield cwt./acre Grain	330	—	—	—	294	100	46	61

* This trial suffered a heavy 'second stage' attack by frit fly which largely accounts for the low yields of grain.

1 cwt./acre of a 2 % powder, was slightly less effective in reducing wireworm population, while the mean yield of grain from the benzene hexachloride treatment was nearly double that obtained with D.D.T.

(*b*) *Application by broadcasting*

In the autumn of 1944 and spring of 1945 benzene hexachloride was applied broadcast on the seed-bed of oats and wheat in seven trials of which four (all on spring oats) were completed. The results of these are presented in Table 4. In an eighth trial the dust was broadcast in the autumn of 1944, on newly ploughed land

which was not cultivated and sown with oats until the following spring. The results of this trial are, therefore, not included for comparison in this series. In every completed trial comparisons were available with combine drilling.

It can be seen that where wireworm populations in untreated control were 1,000,000 per acre or more, the numbers were rather more than halved by 2% benzene hexachloride powder when broadcast at rates varying between $1\frac{1}{2}$ and $6\frac{1}{2}$ cwt./acre, there being no clear evidence of the minimum effective dosage rate.

Combine drilling seemed to have rather similar effects at half the dosage rate per acre. In those trials having 1,000,000 or more wireworms per acre on the untreated plots, all treatments were associated with yields of straw and grain two to five times those of untreated control. It should be noted that in the broadcast treatments the presence of basic slag in the carrier may have contributed to the increased yield, especially in the higher rate of application used in trials nos. 10 and 12. It is of incidental interest to note that in trial 11, Wokingham, undersown seeds (sown broadcast) received striking protection from wireworm attack by the benzene hexachloride treatments, particularly those broadcast. The significance of this observation with relation to its value in ley farming requires no emphasis.

(*c*) *Seed dressings*

Of the six trials on oats laid down near Jealott's Hill, in the spring of 1945, five were completed. In each of these trials the seed dressing technique was compared with combine drilling. In the latter crude benzene hexachloride was used but a specially prepared product containing 40% gamma isomer (Gammexane) in a mixture of the other isomers was used in the seed dressing. The following seed dressings were used: (1) 8% Gammexane on a kaolin base; (2) 20% D.D.T. on a kaolin base.

Both seed dressings were applied at 0·8% on the weight of the seed. The seed was sown at $4–4\frac{1}{2}$ bushels/acre. The results are given in Table 5.

It can be seen that the combine drilled benzene hexachloride was markedly superior to both the Gammexane and D.D.T. seed dressings in its effect on the wireworm population.

In comparing plant populations it is seen that whereas the Gammexane seed dressing was nearly as effective as the combine-drill treatment, that containing D.D.T. was little better than the untreated. In grain yields the combine-drill treatment gave approximately three times the untreated, with smaller increases of approximately two and a half and one and a half times the untreated for the Gammexane and D.D.T. seed dressings respectively.

Duration of effect. In trial no. 19, Warfield, 4% benzene hexachloride preparations in granular and powder forms were broadcast in the autumn of 1944 at about 100 lb./acre, in replicated plots on the ridges of newly ploughed turf. The land was cultivated and sown to oats in the spring of 1945. Plant and wireworm counts were taken a few weeks after sowing. The results are presented in Table 6.

The results show that plant populations on the treated plots a few weeks after sowing were about double those on the untreated, whilst the wireworm populations were reduced to about one-third. Compared with control, the treatments trebled the yield of straw and more than doubled the yield of grain.

TABLE 5. *Effects of Gammexane and D.D.T. applied on seed in the control of wireworm on oats*

		8 % Gammexane seed dressing at 5 oz./bushel	2 % benzene hexachloride dust combine drilled at 1–1½ cwt./acre	20 % D.D.T. seed dressing at 5 oz./bushel	Untreated	Significant difference 0·05	Significant difference 0·01
				Actual data			
Trial 14, Warfield, Berks	Wireworms 1000's/acre	1165	730	1020	1690	220	620
	Plants/1 yd. drill row	18	17	11	10	5·22	7·04
	Yield cwt./acre Straw	24·0	25·9	13·1	3·2	5·61	7·42
	Yield cwt./acre Grain	11·0	12·6	5·1	1·4	2·68	3·55
Trial 15, Binfield, Berks	Wireworms 1000's/acre	750	405	655	1400	625	850
	Plants/1 yd. drill row	35	40	23	17	5·01	7·04
	Yield cwt./acre Straw	32·9	35·9	25·4	24·2	5·73	7·60
	Yield cwt./acre Grain	13·8	15·6	11·4	11·2	2·68	3·55
Trial 16, Wokingham, Berks	Wireworms 1000's/acre	710	560	940	855	490	645
	Plants/1 yd. drill row	21	28	9	6	3·96	5·34
	Yield cwt./acre Straw	19·1	27·4	8·3	9·1	4·44	5·88
	Yield cwt./acre Grain	8·9	14·9	3·9	4·0	2·14	2·82
Trial 17, Wokingham, Berks	Wireworms 1000's/acre	585	540	915	1440	470	620
	Plants/1 yd. drill row	37	39	37	38	5·31	6·83
	Yield cwt./acre Straw	27·9	26·5	18·1	12·3	4·04	5·34
	Yield cwt./acre Grain	11·8	10·8	7·9	5·0	1·71	2·26
Trial 18,* Whistley Mill, Berks	Wireworms 1000's/acre	1270	940	1000	1440	575	765
	Plants/1 yd. drill row	31	34	15	10	6·16	8·20
	Yield cwt./acre Straw	39·3	39·0	23·0	4·6	4·89	6·47
	Yield cwt./acre Grain	17·6	20·4	8·6	1·5	2·35	3·11
Means of all trials	Wireworms 1000's/acre	896	635	906	1369	245	325
	Plants/1 yd. drill row	28	32	19	16	2·86	3·77
	Yield cwt./acre Straw	28·8	31·2	17·6	10·7	2·32	3·07
	Yield cwt./acre Grain	12·6	14·6	7·4	4·6	1·13	1·49
				Data calculated on basis of untreated = 100			
Trial 14, Warfield, Berks	Wireworms 1000's/acre	69	43·2	60·3	100	13	36·6
	Plants/1 yd. drill row	180	170	110	100	52	70
	Yield cwt./acre Straw	750	810	410	100	176	232
	Yield cwt./acre Grain	785	900	365	100	192	254
Trial 15, Binfield, Berks	Wireworms 1000's/acre	53·5	28·9	46·8	100	45	61
	Plants/1 yd. drill row	206	235	135	100	30	41
	Yield cwt./acre Straw	136	148	105	100	24	31
	Yield cwt./acre Grain	123	139	102	100	24	32
Trial 16, Wokingham, Berks	Wireworms 1000's/acre	83	65·5	110	100	57	75·5
	Plants/1 yd. drill row	350	466	150	100	66	89
	Yield cwt./acre Straw	210	302	91	100	49	65
	Yield cwt./acre Grain	223	373	97·5	100	54	70
Trial 17, Wokingham, Berks	Wireworms 1000's/acre	40·6	37·5	63·5	100	33	43
	Plants/1 yd. drill row	97	103	97	100	14	18
	Yield cwt./acre Straw	227	216	147	100	33	44
	Yield cwt./acre Grain	236	216	158	100	34	45
Trial 18,* Whistley Mill, Berks	Wireworms 1000's/acre	88	65·3	69·2	100	40	53
	Plants/1 yd. drill row	310	340	150	100	62	82
	Yield cwt./acre Straw	854	848	500	100	106	140
	Yield cwt./acre Grain	1173	1360	573	100	157	207
Means of all trials	Wireworms 1000's/acre	65·4	46·3	66·2	100	17·9	23·7
	Plants/1 yd. drill row	175	200	119	100	18	24
	Yield cwt./acre Straw	269	291	164	100	22	29
	Yield cwt./acre Grain	275	318	161	100	25	32

* Nitro chalk combine drilled on all plots.

The effect on the crop sown some months after application of the insecticide has again been observed recently, following the sowing of spring oats in 1946 on the site of trial no. 6, Hurst, where benzene hexachloride had been combine drilled with

oats in the spring of 1945. Data obtained in 1945 are compared with those so far collected in 1946 on wireworm and plant populations. The results are recorded in Table 7.

TABLE 6. *Effect of benzene hexachloride broadcast during the autumn previous to the spring sowing of oats in trial no.* 19, *Warfield, Berks*

	4% benzene hexachloride granules at 100 lb./acre	4% benzene hexachloride dust at 100 lb./acre	Untreated	Significant difference 0·05	Significant difference 0·01
			Actual data		
Wireworms 1000's/acre	310	375	935	365	485
Plants/1 yd. drill row	32	35	17	7·28	10·09
Yield cwt./acre Straw	27·5	29·0	9·2	3·96	5·26
Yield cwt./acre Grain	16·0	16·2	7·2	2·74	3·65
			Data calculated on basis of untreated = 100		
Wireworms 1000's/acre	33·3	40	100	39	52
Plants/1 yd. drill row	188	206	100	43	64
Yield cwt./acre Straw	300	315	100	43	57
Yield cwt./acre Grain	222	226	100	38	51

TABLE 7. *The seasonal duration effects of combine drilling benzene hexachloride on spring oats in trial no.* 6

Treatments	1945 Wireworms 1000's/acre	1945 Plants/1 yd. drill row	1945 Yield cwt./acre Straw	1945 Yield cwt./acre Grain	1946 Wireworms 1000's/acre	1946 Plants/1 yd. drill row
			Actual data			
2% benzene hexachloride dust at 1 cwt./acre	1165	34	45·6	25·5	115	49
Untreated	2438	9	14·9	5·0	500	23
Significant difference 0·05	685	5·72	5·9	2·52	235	7·25
Significant difference 0·01	905	7·64	7·8	3·32	295	9·73
			Data calculated on basis of untreated = 100			
2% benzene hexachloride dust at 1 cwt./acre	47·8	378	306	510	23	213
Untreated	100	100	100	100	100	100
Significant difference 0·05	28·2	64	40	50	47	32
Significant difference 0·01	37·2	85	52	66	59	42

DISCUSSION

The experiments described have not closely defined optimum dosage rates. In general, under conditions of heavy wireworm infestation, they have shown on winter wheat and spring oats, two-fold to five-fold increases in grain yield by crude benzene hexachloride (13% Gammexane) broadcast at about 6 lb./acre and combine drilled at about 2 lb./acre. The 8% Gammexane seed dressing applied at approximately 5 oz./bushel of seed increased yield slightly less than the combine drilled crude chemical. It should also be noted that not only was the grain increased in these proportions, but that the treated plots often provided a sample of better and more uniform quality. In general, in highly infested soils reductions in wireworm population to about one-third followed broadcast application of a 2% benzene hexachloride dust at 3 cwt./acre. A reduction to about one-half followed the combine drilling of preparations of the same strength at 1 cwt./acre. The use of a Gammexane seed dressing at a dosage rate in terms of Gammexane per acre,

slightly less than the equivalent dosage used in combine drilling, was associated generally with reductions in wireworm numbers to about three-quarters of the untreated. The experimental error was observed to be considerably higher in data on population than it was on yield but the recorded reductions in wireworm numbers are notable.

The estimated amounts of Gammexane which can be effectively broadcast, combine drilled or applied on the seed lie between 2 and 12 oz./acre and the absence of appreciable differences in the results given by these three methods of application allows a choice to be made on the basis of practical considerations. D.D.T. gave markedly inferior results in seed dressing and combine-drilling trials at 4 and 27 oz./acre respectively in terms of the para-para-isomer compared with corresponding dosage rates of Gammexane of 2 and 4 oz./acre.

The gamma isomer of benzene hexachloride has been shown in earlier experiments against other insects to be 'the active' constituent in the preparations used (Slade, 1945) and this has been confirmed against wireworm in current 1946 trials (to be completed) in which a 90% pure gamma isomer preparation is giving results of an order equal to that of other benzene hexachloride preparations on an equivalent Gammexane basis.

The observed persistent effects of benzene hexachloride (Tables 6 and 7) may be found to be of great value in soils where wireworm is endemic by enabling the desired rotation to be maintained. The protection of the seeds ley as recorded in trial 11 is of particular importance in this connexion.

It is not clear precisely how the observed effects which follow applications of benzene hexachloride are produced. It was clearly evident in every trial that where wireworm attack occurred on the control plots, the treated areas were always protected and showed excellent growth. This protection of the seedlings, invariably followed by increased yields, was not always closely related to the reduction in wireworm numbers and this was most striking with Gammexane seed dressings. These were very effective in plots with high initial wireworm populations which, despite appreciable reduction, were still relatively high after treatment. Immediate or gradual toxic effects may play their part in immobilizing or killing the larvae, and the presence of living wireworms in relatively large numbers in the absence of appreciable attack on the plants may be indicative of a paralysing or repellent effect of the treatment.

While comparable reductions in wireworm populations were obtained with D.D.T., the effects on crop yield were much less marked. It seems possible therefore, that the two chemicals may differ in their action on wireworms in the soil.

Following the trials reported here, much further work is in hand. This has as its object the establishment of minimum dosage rates, investigations of the effects on other crops, and confirmation of the value of selected treatments under a wide range of conditions. Preliminary results from a number of these all confirm the value of Gammexane treatments.

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A DIRECT-SPRAY TECHNIQUE FOR THE BIOLOGICAL EVALUATION OF PYRETHRUM-IN-OIL INSECTICIDES FOR USE AGAINST STORED PRODUCT INSECTS IN WAREHOUSES

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(With Plate 7 and 6 Text-figures)

A direct-spray technique for the biological assay of pyrethrins in Shell oil P 31 is described, together with the experiments leading to its adoption. The technique is such that film effects are reduced to the minimum practicable, but it is otherwise as similar as possible to the film technique described by Parkin & Green (1943). Methods for statistical analysis of the results are discussed. For the biological assay of pyrethrins in Shell oil P 31, the direct-spray method is slightly superior to Parkin & Green's film method, but both techniques should have their uses in general experimental work.

Introduction

A solution of pyrethrins in a heavy, highly refined, mineral oil, Shell oil P 31, is now used extensively as a spray for the control of insects infesting stored products. During these spraying operations the insects receive doses of insecticide in one or more of three ways: viz. (1) as a film, when the insects come into contact with insecticide previously deposited on the surfaces on which they walk; (2) as a mist, the droplets of which are impacted on to the insects by slow air currents or by sedimentation, or by movement of the insects, especially flight; and (3) as a direct spray, when insecticide is impacted on the insects by droplet movement imparted by the spray gun.

Film effects are largely relied upon for killing insects infesting food or food stores, but, in view of the low toxicity of films formed by pyrethrum in P 31 on materials commonly encountered in practice (see Parkin & Hewlett, 1946), direct-spray effects must often play an important part in these control operations. It was therefore decided that some direct-spray method for the laboratory evaluation of insecticides, additional to the film method of Parkin & Green (1943), would be useful.

The object of the investigation described here was to provide a method which would measure direct-spray effects only, but which would be otherwise as similar as possible to the film technique devised by Parkin & Green, so as to permit comparison of results obtained by the two procedures. As many as possible of the factors which might influence mortality were standardized as in the film technique: of the remainder, some were standardized arbitrarily, and the rest were fixed in the light of

experimental work. In particular, the standard insecticide employed during this investigation was 1·6% w/v total pyrethrins in P31, as used by Parkin & Green.

To avoid frequent repetition, a description of the direct-spray technique precedes an account of its development.

A comparative direct spray technique

A. *Description of technique*

(1) *Method for breeding test insects*

The test insects are adult *Tribolium castaneum* Herbst bred by the technique of Parkin & Green (1943), with the following slight modifications: the breeding temperature is 25° C.; each main culture is started with seventy beetles 0–2 weeks old; these beetles are sieved off 5 weeks later and the F_1 adults removed at fortnightly intervals thereafter.* These modifications in culturing procedure were introduced because of the shorter life history and greater fecundity of the beetles at present in use at the Pest Infestation Laboratory in comparison with the beetles used by Parkin & Green.

(2) *Treatment of the insects before spraying*

The treatment of the insects before being sprayed is the same as that before exposure to films (see Parkin & Green, 1943), except that the beetles are starved overnight at 25° C. instead of at 24° C.*

(3) *Spraying of the insects*

(*a*) *Spraying apparatus.* The spraying apparatus, shown in Pl. 7, fig. 1, is an improved form of the spraying tower designed by Potter (1941). It is fitted with atomizing nozzle P.I.L. no. AN2 or AN3 (see Hewlett, 1946). Other modifications were introduced by Parkin & Green (unpublished). The excess spray discharged from between the lower flange of the tube and the spray table is removed by a current of air passing downwards within a shield around the bottom of the tower, through a grating under the tower, and so along a duct to waste. The air current is maintained by a fan in the duct, and is just sufficient to remove all the excess spray. The shield is 18 in. in diameter and 17 in. in height. When insects are being sprayed, the bottom of the shield rests on the bench on which the spraying tower is mounted (see Pl. 7, fig. 1), so that the top of the shield is $2\frac{1}{2}$ in. above the flange on the bottom of the tube of the tower. When insects are put on or taken off the spray table, the shield is lifted, and held in position by means of cords and counter-weights.

The nozzle setting is $\frac{1}{2}$-turn (see Hewlett, 1946), and the gap between the spray table and the lower flange of the tube $\frac{15}{32}$ in.

The temperature of the air stream in which the insects are sprayed is 20–21° C., and while being sprayed the insects are exposed to electric light of low intensity.

* These modifications have been introduced into the film test of Parkin & Green as now carried out at the Pest Infestation Laboratory. The insects are now also exposed on the films at 25° C.

(*b*) *Enclosure of the insects during spraying*. While being sprayed the insects are enclosed within a duralumin ring, highly polished on its internal surface, standing on a Whatman no. 1 filter-paper. The filter-paper is supported by a circular duralumin plate, and the portion of the filter-paper external to the duralumin ring is covered by a brass mask. The duralumin plate, etc., are placed on a piece of thick cardboard covered by a filter-paper. Pl. 7, fig. 2*a*, shows the separate parts of the apparatus, and Pl. 7, fig. 2*b*, the apparatus set up for confining insects. When putting the apparatus on, or taking it off, the spray table, the cardboard disk is held with forceps. The dimensions of the different parts are as follows:

Duralumin ring: internal diameter, 6·0 cm.; external diameter, 6·3 cm.; depth, 0·8 cm.
Brass mask: internal diameter, 6·35 cm.; external diameter, 7·7 cm.; depth, 0·3 cm.
Filter-paper covering duralumin plate: diameter, 7·0 cm.
Duralumin plate: diameter, 6·9 cm.; thickness, 0·17 cm.
Cardboard disk: diameter, 8·5 cm.

(*c*) *Insecticide*. The standard insecticide, 1·6% w/v total pyrethrins in Shell oil P31, is prepared by diluting a pyrethrum-in-P31 concentrate containing approximately equal amounts of pyrethrin I and pyrethrin II, and filtering through Whatman no. 544 filter-paper. The insecticide is used on the day on which it is made up, and when used its temperature should be 20–21° C. The concentrate should contain an antioxidant, e.g. 2·5% by volume of a 10% w/v solution of pyrocatechol in ether. If desired, the amount of oleoresin may be controlled as described by Martin (1943). The specification of P31 has been given by Robinson (1942).

(*d*) *Estimation of dosage*. The dose of insecticide applied to a batch of insects is measured by the weight per unit area which would be deposited on the filter-paper within the duralumin ring enclosing the insects, if insects were not present. For instance, the deposit on the filter-paper within the ring, as a result of spraying 2 ml. of insecticide through the atomizing nozzle when insects are absent, is taken as a measure of the dose received by the insects when 2 ml. of insecticide are sprayed under similar conditions. It is therefore necessary to relate, in the absence of insects, the volume of insecticide introduced into the reservoir of the atomizing nozzle to the resultant deposit of insecticide within the duralumin ring.

Above a 'critical' level of deposit, which must be determined for the particular nozzle used, but which can be expected to be about 6 mg./10 sq.cm.,* the deposit on the filter-paper within the ring is, for solutions of pyrethrins in P31, directly proportional to the volume of liquid introduced into the atomizing nozzle. The ratio of the volume of liquid introduced into the reservoir of the nozzle to the deposit (V/D ratio) is determined for each insecticide at a deposit well above the critical level. If, when spraying insects, the deposit estimated from the liquid volume by

* Deposits are measured in mg./10 sq.cm., rather than mg./sq.cm., to avoid negative log-deposits in the statistical analysis of results.

the V/D ratio is above the critical level, this estimated deposit may be taken as the true deposit, but if this estimated deposit is below the critical level, it must be corrected by means of a calibration curve, similar to Text-fig. 2, appropriate to the particular nozzle used. The calibration curve can be expected to be applicable during the use of any concentration of pyrethrins in P31 up to 3·5%, the highest concentration tested.

(*e*) *Spraying procedure.* The spraying pressure is 20 ± 0·1 cm. of mercury. After each spraying the nozzle is washed out by spraying about 1 ml. of carbon tetrachloride, and air is allowed to pass for about 1 min. afterwards. Insecticide is put into the nozzle reservoir by means of a hypodermic syringe: if two or more syringes are used in conjunction, they must be calibrated, for the weights of insecticide delivered by different syringes, though marked as the same volumes on the syringes, may differ by more than 10%. Between sprayings the duralumin ring and brass mask are cleaned, and the filter paper on which the duralumin ring stands is renewed.

Before spraying batches of insects with a given insecticide, the V/D ratio is determined. The insect enclosure, containing no insects, is sprayed three times, the same volume of insecticide being introduced into the nozzle reservoir each time, and the weights of spray deposited on the area of filter-paper within the ring determined by weighing. If the weights of spray differ by less than ± 1% from the mode, the V/D ratio is calculated from the average of the weights: if the difference is greater, the nozzle is cleaned, or the apparatus otherwise adjusted to improve replication. Insects are sprayed in batches of fifty, the liquid volume being adjusted according to the deposit required. Each fifth spraying is made a check of the V/D ratio: if the weight of spray is not within the range formerly observed, the V/D ratio is redetermined.

(4) *Treatment of the insects after spraying*

When a batch of insects has been sprayed, the enclosure, containing the insects, is removed from the spraying tower. The brass mask is lifted off, and a clean brass ring (not shown in Pl. 7, fig. 2), 6·5 cm. in internal diameter and 0·8 cm. in height, is placed round the outside of the duralumin ring: the duralumin ring is then removed, and the insects are gently swept off the filter-paper on which they have been sprayed into a container.

Each container consists of a glass ring 6 cm. in diameter and 2 cm. in height, standing on a clean Whatman no. 1 filter-paper 7 cm. in diameter. The sprayed insects remain on the filter-paper, and when they have been put into the container a piece of wire gauze, of 20 or 30 meshes to the inch, is laid over the top of the glass ring to prevent any insect from escaping by flying out. Less than 10 min. after being sprayed, the batches of insects in the containers are transferred to a constant temperature room maintained at 25° C. and 70% R.H., where they remain until observations on them are complete. They are kept in the dark for the greater part of the period between spraying and observation.

As in the film test of Parkin & Green (1943), all glassware should be scrupulously clean, and free from soap, which is liable to remain on the surface after washing.

(5) *Observations of results*

The insects are left at 25° C., and the numbers dead are counted 6 and 9 days after spraying. The beetles are examined at 25° C. under a low-powered binocular microscope in the same way as in the film technique, i.e. *in situ* on the filter-papers on which they are confined after spraying. Dead insects, i.e. those neither moving spontaneously nor responding by reflex movement when touched with a brush, are removed and counted. Living beetles are disturbed as little as possible.

B. *Analysis of results*

The toxicities of insecticides may be compared on the results of either count. The results can be analysed statistically by the methods of Bliss (1935*a*, *b*, 1938), with weighting coefficients taken from Finney (1944) to allow for control deaths. Data obtained by the direct spray method described here should normally be homogeneous with respect to goodness of fit to provisional regression lines; so that usually, during the comparison of two insecticides, the slope and position of their regression lines can be compared by direct χ^2 tests (Bliss, 1935*b*). If the data are heterogeneous and it is desired to proceed with the comparison, *t*-tests must be used.

Bliss (1938) gives an expression by which the standard error of the log-deposit or log-concentration corresponding to any given mortality can be found. The order of magnitude of the standard error of the log-LD 50 may be obtained by the method of Litchfield & Fertig (1941). Other useful statistical procedures are given by Miller *et al.* (1939), e.g. a χ^2 test for agreement of the slopes of more than two regression lines, and a χ^2 test for agreement between the values for the relative potency, *M*, obtained in different experiments.

C. *Limits of the test*

It has been shown that in nineteen tests out of twenty it should be possible to distinguish 1·35% w/v pyrethrins in P 31 from 1·6% at a 1% significance level, and to distinguish 1·4% from 1·6% at a 5% significance level, when 400 insects are used to determine each regression line, covering a 20–95% mortality range. The test could be made more sensitive by using larger numbers of insects.

Development of the direct-spray technique

Before starting experimental work, the factors which might influence the effect of the pyrethrins on the test insects were listed, as follows:

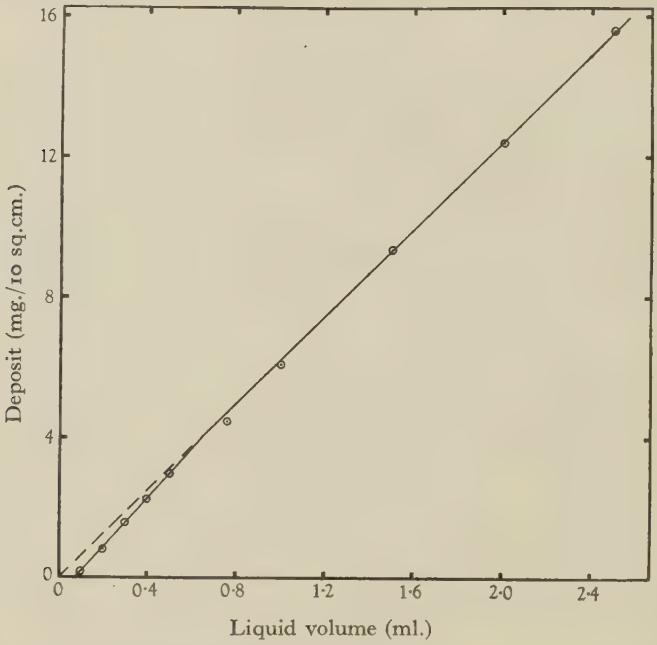
(1) *Test insects.* Species and method of breeding.

(2) *Treatment of the insects before spraying.* Period of starvation, disturbance during counting, temperature, humidity, and light intensity.

(3) *Conditions during spraying of the insects.* Spraying apparatus, method of enclosure of the insects, concentration of pyrethrins in the insecticide, carrier, amount of insecticide applied to the insects (dosage), temperature, humidity, and light intensity.

(4) *Conditions after spraying of the insects.* Method of transferring sprayed insects to containers, type of container, foothold in containers, period between spraying and observation of results, temperature, humidity, and light intensity.

The factors grouped under (1) and (2), the concentration of pyrethrins in the insecticide, and the carrier, were standardized as in the slightly modified version



Text-fig. 1. The relation between the volume of P31 introduced into the reservoir of the atomizing nozzle and the deposit on the filter-paper within the duralumin ring.

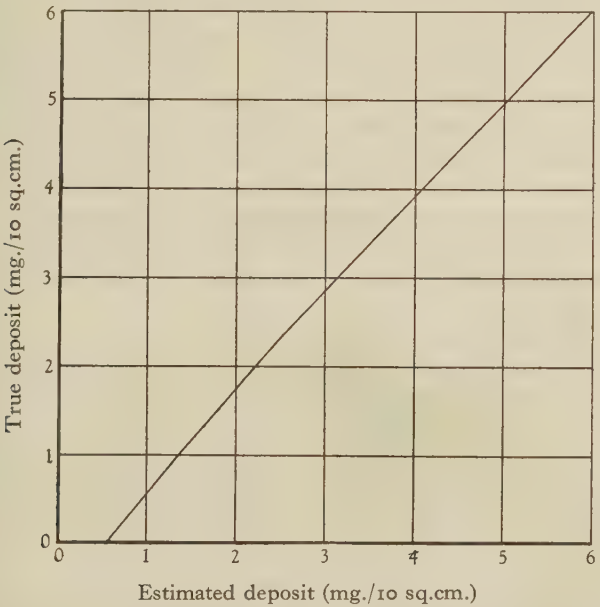
(see p. 358) of Parkin & Green's film technique. As its performance was satisfactory (see Hewlett, 1946), the modified form of the spraying tower described by Potter, (1941) fitted with atomizing nozzle P.I.L. no. AN2, was selected as a suitable spraying apparatus. The sprayed insects were kept at 25° C. and 70% R.H., so that the insecticide could exert its toxic effect on them under conditions similar to those in the film technique. Of the factors remaining, some were standardized arbitrarily and the rest in the light of experiment.

It was decided to assess the effect of the pyrethrins on the *T. castaneum* by classifying the treated and control beetles as dead or alive, because Parkin & Green did

likewise, having found other types of classification unsatisfactory. By the method already described, dead beetles could be distinguished from the living easily and objectively.

A. *Enclosure of the insects during spraying*

Fifty beetles could be confined within a ring 6 cm. in diameter without apparent overcrowding, and, provided that the internal surface of the ring was highly polished, a ring 0·8 cm. in depth was sufficiently deep to prevent their escape. In still air the majority of the beetles collected near the periphery of their enclosure,



Text-fig. 2. The relation between the deposit estimated from the V/D ratio and the true deposit for true deposits of 0–6 mg./10 sq.cm.

but while being sprayed they spread much more evenly over the area enclosed by the ring, as a result of stimulation by the air current in the spraying tower. The distribution of spray deposited within the ring was found to be uniform (cf. Hewlett, 1946). The function of the brass mask will be explained in relation to the measurement of dosage.

B. *Measurement of dosage*

Preliminary experiments showed that it was not practicable to measure dosage by the weight of insecticide adhering to sprayed insects. Knowing the weight of a batch of insects before spraying, and that of the container to which the batch would be transferred, the weight of insecticide adhering to the insects after spraying was

found from the weight of the container + sprayed insects. Although batches of insects were sprayed under the same conditions, the weights of the adherent insecticide varied considerably from batch to batch. This variation was almost certainly caused by a rapid blotting of insecticide from the insects, during and immediately after spraying, on to the filter-papers on which they were sprayed.

Insects lost insecticide to their containers for the first 2–3 hr. after spraying, after which the amount of insecticide adhering to the insects appeared to remain constant. Attempts were made to weigh the insecticide adhering to the insects 3 hr. after spraying, but these attempts were unsuccessful, because insects sprayed with pyrethrum lost 5% or more of their body weight during the first 3 hr. after spraying. Unsprayed insects, and insects sprayed with P31 alone, lost less than 0·5% of their weight under the same conditions, so that the 5% loss of weight is attributable to the physiological effect of the pyrethrum. This point will be further investigated later.

After these preliminary experiments, the method finally adopted for measuring dosage (see p. 359) was investigated. The function of the brass mask (see pp. 359, 360) was to prevent the deposition of spray on that part of the filter-paper outside the duralumin ring. If the mask was not used, insecticide was deposited on the extreme edge of the filter-paper, but not immediately outside the duralumin ring; in which circumstances the total weight of insecticide deposited on the paper was not representative of the dose received by the insects.

Two experiments, the results of which were in complete agreement, were carried out to determine the relation between the volume of P31 introduced into the reservoir of the atomizing nozzle and the deposit on the filter-paper within the duralumin ring. The results of the more comprehensive experiment are shown in Text-fig. 1, from which it is evident that above a deposit of about 6 mg./10 sq.cm. the deposit is directly proportional to the volume of P31, but below 6 mg./10 sq.cm. the deposit falls below the value predicted on the basis of such proportionality. The relation between liquid volume and deposit was also determined for 1·6 and 3·5% pyrethrins in P31. The relationships were very similar to that when P31 was used, but the V/D ratios were both slightly lower than that for P31, the ratio for 3·5% pyrethrins being lower than that for 1·6%. The V/D ratio for a given solution was found to vary by as much as ±2% on different days, so that deposit could not be estimated from a number of calibration curves for different concentrations of pyrethrins. While deposits above 6 mg./10 sq.cm. could be estimated by determination of the V/D ratio on any given occasion, the estimation of deposits below 6 mg./10 sq.cm. still presented difficulties. However, it was found that the relation between true deposits below 6 mg./10 sq.cm. and apparent deposits estimated from V/D ratios was independent of the value of the V/D ratio and of the solution used. This relationship is shown in Text-fig. 2.

These considerations led to the method for the estimation of dosage finally adopted (see p. 359). As an example, if the V/D ratio was 0·160 ml./mg./10 sq.cm., and the volume of liquid introduced into the reservoir was 0·5 ml., the first estimate

of the deposit, by the V/D ratio, would be 3·13 mg./10 sq.cm., which would be corrected to 2·95 mg./10 sq.cm. by means of Text-fig. 2.

C. *Comparison of different concentrations of pyrethrins by the standard technique*

Preliminary experiments with convenient ranges of deposits (2–30 mg./10 sq.cm.) showed that the mortalities were too low for the comparison of different pyrethrin concentrations between 0·8 and 1·6% if the period between spraying and observation was as short as 3 days. Periods of 6 and 9 days, however, gave suitable ranges of mortality, and these periods were otherwise appropriate, since the pyrethrum could then exert its effect on the insects for the same time as in the film technique of Parkin & Green (1943). The control mortality, after beetles had been starved for 9 days, was rarely more than 2%.

Three concentrations of pyrethrins, 0·8, 1·2, and 1·6%, were compared using the standard technique. The 6-day results are shown graphically in Text-fig. 3,* in which probit mortality has been plotted against log-deposit, and lines fitted statistically. The data fitted straight lines reasonably well. The lines differed significantly in position but not in slope. The 9-day results were similar.

In view of these results, the standard technique, at this stage in the investigation adopted provisionally, was *prima facie* satisfactory. Further experimental work was therefore undertaken to make certain that the technique was sound, or to indicate how it might be improved.

D. *Further investigation of the standard technique*

(1) *Effect of oil alone*

Deposits of 30 mg./10 sq.cm. of P31 alone were found non-toxic to *Tribolium castaneum*. Oil effect was therefore absent within the range of deposits likely to be used.

(2) *Effect of different methods for transferring sprayed insects to containers*

The mortalities by the standard procedure (method A) of transferring the insects to the containers for observation, using the clean brass ring, were compared with those obtained when the insects were swept into the containers by the duralumin ring, which was wet with insecticide (method B).

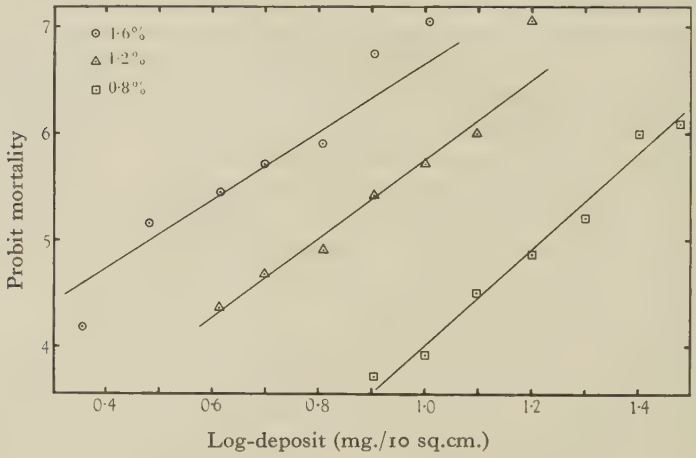
The data obtained were examined by plotting probit mortality (corrected for control mortality) against log-deposit, and the 9-day results are shown graphically in Text-fig. 4. The 9-day results were more homogeneous than the 6-day, but both indicated that by method A the slopes of the regression lines were higher and the LD 50's lower than those obtained by method B, although the differences were not large. It was concluded that the insects picked up doses of insecticide from the duralumin ring in method B, and that the doses of insecticide received by individual

* In Text-figs. 3–5 each point represents an observation based on a batch of forty-eight to fifty insects.

insects of a batch were more variable by method B than by method A. Method A, i.e. use of the clean brass ring, was therefore employed in all further experiments.

(3) *Film effect during the observation period*

When sprayed insects were confined on a clean filter-paper, some insecticide was transferred from the insects to the paper. Insects might have remained in contact with insecticide sorbed by the paper, and so have received a dose of insecticide by film effect. To discover whether the substrate on which the sprayed insects were confined could influence the mortalities observed, the standard technique was compared with one in which each batch of insects was confined within a glass ring on a 3-in. square of ground glass. The results are shown in Table 1.



Text-fig. 3. Comparison of the 6-day mortalities given by 0·8, 1·2 and 1·6% pyrethrins in P31.

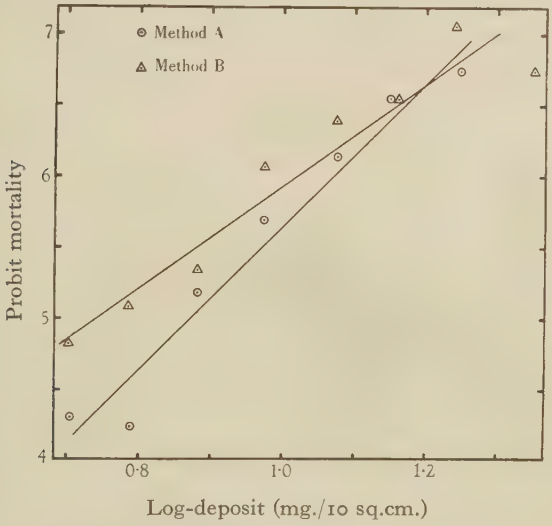
Table 1. *Comparison of the effects of confinement of sprayed* Tribolium castaneum *on Whatman no. 1 filter-paper and on ground glass. Each figure is derived from fifty insects. Insecticide: 1·6% pyrethrins in P31*

Deposit (mg./10 sq.cm.)	Mortality %: 6 days* Filter-paper	6 days* Glass	9 days* Filter-paper	9 days* Glass
2·49	16	14	20	18
3·21	36	64	42	72
3·92	34	82	44	88
4·99	52	78	64	82
6·36	66	90	78	94
7·99	80	86	86	96
Control†	0	1	2	1

* Period between spraying and observations. † Figures from 100 insects.

The data of Table 1 show that at each deposit the mortality on glass was, with one unimportant exception, higher than that on filter-paper, proving that the substrate on which the insects are confined after spraying can influence the mortalities observed.

The next step was to find out whether a film effect occurred when the insects were confined on Whatman no. 1 filter-papers. Batches of insects were sprayed respectively with about 10 mg./10 sq.cm. of 1·6% pyrethrins, 15 mg./10 sq.cm. of 1·2% pyrethrins, and 30 mg./10 sq.cm. of 0·8% pyrethrins, i.e. the highest deposit of each pyrethrin concentration likely to be used during direct spraying. The sprayed insects were transferred to weighed Whatman no. 1 filter-papers, from which they were removed $2\frac{1}{2}$ hr. later, when the papers were weighed again. The



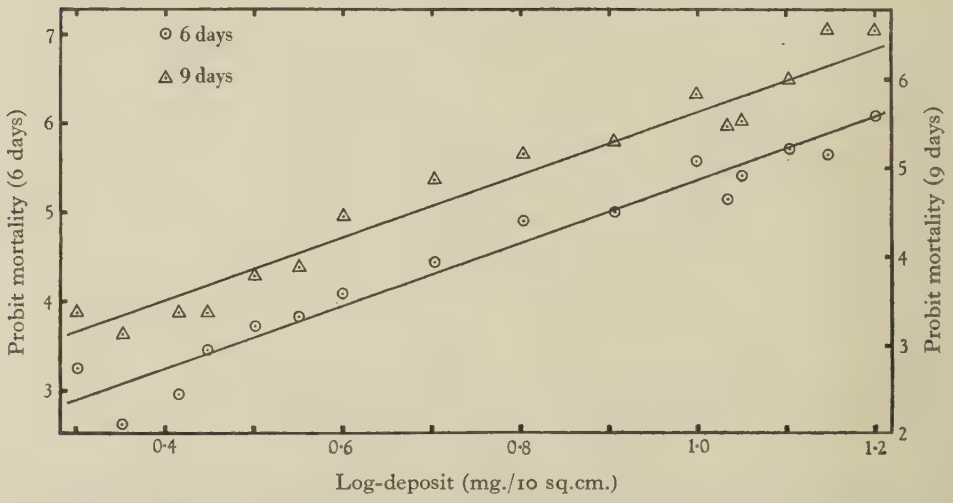
Text-fig. 4. Comparison of the 9-day mortalities obtained when sprayed insects were swept into containers with a clean brass ring (method A) and a duralumin ring wet with insecticide (method B).

weight of insecticide blotted off the insects by the filter-papers was approximately the same for each pyrethrin concentration, i.e. about 3 mg. on each filter-paper. The area that had sorbed insecticide on each filter-paper was then estimated planimetrically as about 16 sq.cm. The film of insecticide with which the insects might be in contact after being sprayed was therefore equivalent to a film formed by a deposit of less than 2 mg./10 sq.cm., since not all the insects would be in contact with the film. After spraying, the film effect on the directly sprayed insects was therefore equivalent to confinement on a film formed by a deposit of less than 2 mg./10 sq.cm. However, when batches of unsprayed insects were confined on

films formed on Whatman no. 1 filter-papers by 5–6 mg./10 sq.cm. of 1·6% pyrethrins, the mortalities after 6 days were about 2% and after 9 days about 16%. Since 5–6 mg./10 sq.cm. is a deposit considerably higher than that with which insects are likely to be in contact after being directly sprayed, the film effect on Whatman no. 1 filter-papers in the provisional direct spray technique was considered negligible; especially as only a proportion of directly sprayed insects (on the basis of areas, about 50%) remain in contact with insecticide sorbed on the filter-paper.

(4) *The deposit-mortality relation*

An experiment was carried out to discover whether the probit mortality-log-deposit relation for fixed concentration was linear over a range of mortalities wider



Text-fig. 5. The relation between probit mortality and log-deposit for a wide range of deposits of 1·2% pyrethrins.

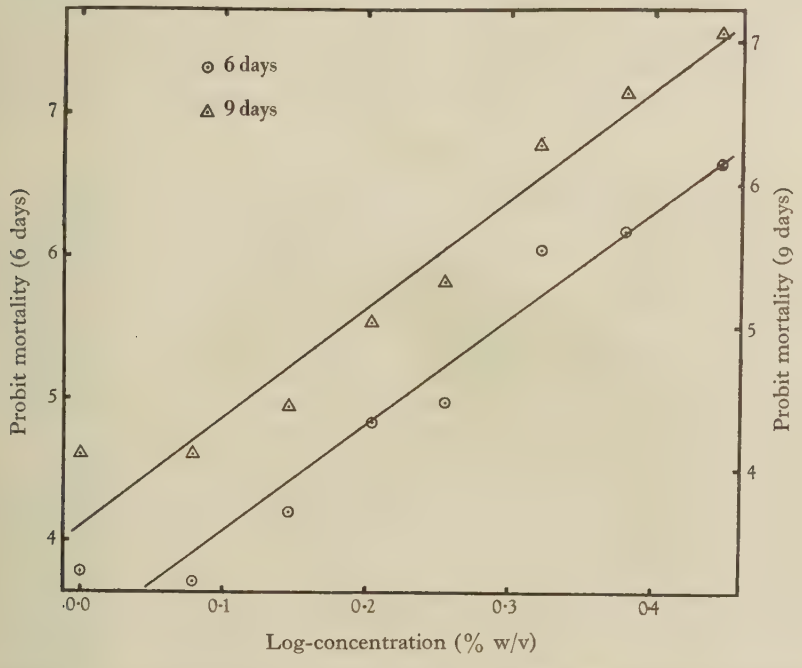
than that of the data previously collected. Insects were sprayed with 1·2% pyrethrins, using a wide range of deposits of which the majority were intended to give mortalities of 0–30 or 70–100%: 1·2% pyrethrins was used in preference to 1·6%, because deposits low enough for the lower mortality range could be more accurately sprayed with the former concentration.

The mortalities in the 70–100% range were not so high as had been hoped. The results are shown graphically in Text-fig. 5, from which it is evident that there was no systematic departure from linearity in the probit mortality-log-deposit relation between 3 and 6·5 probits. The experiment was not repeated because data for the higher mortality range were obtained in other experiments. The data indicated

generally that the probit mortality-log-deposit relation was linear between 3 and 7 probits (2–98% mortality) for 1·2 and 1·6% pyrethrins.

(5) *The concentration-mortality relation*

The relation between probit mortality and log-concentration was investigated by spraying batches of insects with different concentrations of pyrethrins at a deposit of 3·5 mg./10 sq.cm.: this deposit gave approximately 50% mortality for 1·6% pyrethrins. Concentrations of 1·0, 1·2, 1·4, 1·6, 1·8, 2·1, 2·4, and 2·8% were employed, and in all sixteen batches of fifty insects were sprayed, two with each concentration.



Text-fig. 6. The relation between probit mortality and log-concentration of pyrethrins in P31 at a deposit of 3·5 mg./10 sq.cm.

The mean mortalities for each concentration were calculated, the means corrected for control mortality, and the corresponding probit values plotted against log-concentration, as shown in Text-fig. 6. The sets of points for 6 and 9 days fitted two straight lines well, except the points for 1·0% pyrethrins. Between 4 and 6·5 probits (16–93% mortality) probit mortality appeared to be linearly related to log-concentration at a deposit of 3·5 mg./10 sq.cm.

(6) *Limits of accuracy of the technique*

In comparing an 'unknown' insecticide, i.e. an insecticide to be assayed, with the standard (1·6% pyrethrins), it was envisaged that probit mortality-log-deposit regression lines would be obtained for each. Provided that the two lines did not differ significantly in slope, the unknown could be pronounced, within given fiducial limits, not inferior or inferior to the standard by comparison of the positions of the regression lines by the methods of Bliss (1935*a*, *b*, 1938). If in a given test the unknown and the standard did not differ significantly in toxicity, the unknown would be judged more toxic than a concentration of pyrethrins lower than, but just distinguishable from, 1·6%. This concentration will be referred to as the limiting concentration. It is, of course, an indication of the quality of the method of assay, for the better the method the nearer to the standard will the limiting concentration be.

To find the limiting concentration, the standard was compared with a concentration (referred to as the 'comparator') lower than the standard, but significantly distinguishable from it, and the limiting concentration calculated from the results. The limiting concentration is given by

$$c_u = c_1 - \frac{\rho(c_1 - c_2)}{a_1 - a_2 - b_c(\bar{x}_1 - \bar{x}_2)} \sqrt{[V(a_1) + V(a_2) + (\bar{x}_1 - \bar{x}_2)^2 V(b_c)]}, \qquad (1)$$

where c_u = log-limiting concentration, c_1 = log-standard concentration, c_2 = log-comparator concentration, and ρ is taken from the tables of Neyman & Tokarska (1936).

The other symbols have the same meaning as given by Bliss (1935*a*, *b*). The value of ρ depends upon the fiducial limits adopted and the power of the test. For instance, if the significance level was 5% and the power 0·90, it would be possible to distinguish the limiting concentration (given by equation (1)) from the standard in nine tests out of ten at the 5% significance level. Along the top of their tables, Neyman & Tokarska list a quantity P_{II} equal to the difference between the power and 1. The degrees of freedom of ρ are given by the total number of points determining the two regression lines less 3.

Equation (1) is based upon an imaginary *t*-test, comparing the positions of regression lines given by the standard and limiting concentrations. In its derivation, it was assumed that the regression lines for the standard and comparator did not differ significantly in slope, and that the relation between probit mortality and log-concentration was linear, at least between the standard and comparator concentrations. The former assumption could be tested by the methods described by Bliss (1935*b*), while the latter was justified by the experimental work of the preceding section of this paper. It should be emphasized that equation (1) is correct only if the regression lines given by the standard and comparator differ significantly in position.

The limiting concentration will vary according to the numbers of insects exposed to the insecticides, the mortalities, the significance level, and the power of the test. In comparing the standard with an unknown, it was convenient to use eight batches

of fifty insects to determine each regression line, and to choose doses in a geometric series to give mortalities covering a range of 20–95%. In comparing the standard and the comparator, therefore, the same number of insects was used, and the doses adjusted in the same way. Significance levels of 5 and 1%, and a power of 0·95, were selected.

The comparator concentration should not differ very greatly from that of the standard, in case the technique inherently gives more homogeneous results at one level of concentration than at another. 1·3% pyrethrins was therefore compared with 1·6% in three separate experiments. Equation (1) may be expressed in the form

$$c_u = c_1 - K\rho, \qquad (1a)$$

where K is a constant. If the value of K is known, the value of c_u can be found for any significance level and power. The estimates of K obtained experimentally were as follows:

Exp.	6 days*	9 days*
23	0·0145	0·0185
25	0·0167	0·0153
27	—	0·0176

* Period between spraying of insects and observation results.

The regression lines for 6 days, Exp. 27, differed significantly in slope ($P<0·01$), so that the corresponding value of K could not be calculated. In no other case did the lines differ significantly in slope ($P>0·2$).

For a power of 0·95 and $P=0·05$ and 0·01, the limiting concentrations (% w/v) of pyrethrins were as follows:

	$P=0·05$		$P=0·01$	
Exp.	6 days	9 days	6 days	9 days
23	1·425	1·380	1·379	1·343
25	1·402	1·416	1·348	1·368
27	—	1·390	—	1·336

There was no evident systematic difference between the limiting concentrations calculated from the 6-day and 9-day results. Mean values for the limiting concentrations were not calculated, but that for $P=0·05$ would be very near 1·4%, and that for $P=0·01$ very near 1·35%. It was considered that these limits of accuracy for the test were satisfactory.

(7) *Statistical analysis of data*

The data obtained in the comparison of 0·8, 1·2, and 1·6% pyrethrins (Text-fig. 3), and in the determination of the limiting concentrations, were analysed statistically by the methods of Bliss (1935*a*, *b*; 1938). The results of χ^2 tests for the goodness of fit of the data to the regression lines are given in Table 2.

The separate and total values for χ^2 in Table 2 indicate that the points fitted the regression lines satisfactorily. The χ^2 values for 6 days show no consistent tendency to be higher or lower than those for 9 days, although the total χ^2 for 6 days is lower than that for 9 days.

Tests were also carried out to discover whether the regression lines obtained from the same data were consistent in slope. The values of χ_b^2, calculated by means of equation (6) of Miller *et al.* (1939), were 7·66 and 7·50 for the 6-day and 9-day results respectively, each with eight degrees of freedom. The corresponding values of P were approximately 0·47 and 0·48, so that the lines agreed satisfactorily in slope. The values of b_c were 3·83 and 3·94 for the 6-day and 9-day results respectively.

The statistical tests as a whole showed that there was little to choose between the 6-day and 9-day results.

TABLE 2. *The fit of points to regression lines, shown by values of* χ^2 *and* P

Pyrethrin concentration (% w/v)	n	6 days*		9 days*	
		χ^2	P (approx.)	χ^2	P (approx.)
0·8	5	2·173	0·82	6·335	0·28
1·2	5	3·145	0·68	2·564	0·77
1·3	6	1·544	0·96	5·365	0·50
1·3	6	10·723	0·10	10·621	0·10
1·3	6	4·930	0·55	1·194	0·98
1·6	5	5·583	0·35	5·829	0·33
1·6	6	5·866	0·44	10·177	0·12
1·6	6	3·740	0·71	3·280	0·77
1·6	6	5·474	0·48	8·139	0·23
Totals	49	43·178	0·76	53·504	0·31

* Period between spraying of insects and observations of results.

The conclusions drawn from the investigation of the provisionally adopted standard technique may now be summarized. The oil solvent, Shell oil P31, exerted no toxic effect. The method of transference of sprayed insects to containers for observation could not safely be simplified. The relationships between probit mortality and log-deposit, and between probit mortality and log-concentration were linear over wide ranges of mortality. The standard and limiting concentrations were reasonably close together. The technique gave statistically satisfactory results; for the data were homogeneous, as shown by the goodness of fit to regression lines, and the regression lines for different concentrations were parallel within the limits of sampling error. In view of these conclusions, the technique was considered satisfactory.

DISCUSSION

In the direct-spray technique described insects receive little insecticide by film action. They are transferred from the filter-papers on which they have been sprayed to clean filter-papers, and the amount of insecticide blotted off the insects on to the latter is too small to exert an important toxic effect. In practice, on the other hand, insects exposed to a direct spray will often remain on the surface on which they have been sprayed, and, if the surface is such that it will support a toxic film, the insects will receive an additional dose of insecticide by film action. If the surface does not

support a toxic film (see Parkin & Hewlett, 1946), the conditions of dosage will be very similar to those in the direct-spray test. The conditions of dosage in the direct-spray test are therefore like those of a limiting case in practice.

In the direct-spray technique beetles lose much of the insecticide sprayed on to them to the filter-papers on which they are confined, just as flies sprayed in a chamber lose insecticide to their surroundings (see David, 1946). A high proportion of that not lost by the beetles is retained probably because it has been drawn by capillary action into the intersegmental folds, into the cuticular folds of the limb joints, under the elytra, etc. This capillary effect can easily be demonstrated: a small drop of insecticide placed at the junction of the prothorax and mesothorax is quickly drawn into the intersegmental fold. Insecticide may well be more poisonous in folds of the integument than on the general surface of an insect, because in the former situation it will generally be in contact with thinner cuticle, likely to be less resistant to insecticidal penetration (see Wigglesworth, 1942), than in the latter.

Since, using pyrethrins in P31, probit mortality is linearly related to log-concentration and log-deposit, and since the probit mortality-log-deposit regression lines for different concentrations are parallel, data for this insecticide could be fitted to a probit plane (see Finney, 1943), at least over certain ranges of concentration and deposit. The equations of the planes for 6 and 9 days would be approximately

$$6 \text{ days:} \quad Y = 1{\cdot}3 + 8{\cdot}0\, x_1 + 3{\cdot}8\, x_2, \tag{2}$$

$$9 \text{ days:} \quad Y = 1{\cdot}5 + 8{\cdot}0\, x_1 + 3{\cdot}9\, x_2, \tag{3}$$

where Y = probit mortality, x_1 = log-concentration, % w/v, and x_2 = log-deposit in mg./10 sq.cm.

It has been shown that, using 400 insects per concentration and 20–95% mortality, in nineteen tests out of twenty, 1·35 and 1·4% pyrethrins should be distinguishable from 1·6% at 1 and 5% significance levels respectively. For a given value of ρ, over certain ranges of concentration antilog $(c_1 - c_u)$ will be constant, that is to say the ratio of the standard to the limiting concentration will be constant. 1·6/1·35 = 1·18 and 1·6/1·4 = 1·14. Hence between concentrations of, say, 1·2 and 2%, pyrethrin concentrations differing by factors of 1·18 and 1·14 should in nineteen tests out of twenty be distinguishable at 1 and 5% significance levels respectively.

It would be possible to simplify the direct-spray test by comparison of the mortalities given by replication at a fixed deposit. However, until it has been shown that there is a reasonable expectation that the slope of the probit mortality-log-deposit regression line for the unknown insecticide will not differ significantly from that for the standard, such a simplification is unjustifiable.

As well as the direct-spray technique described here, three other techniques are available for the biological assay of pyrethrum in a heavy oil, viz. the film technique of Parkin & Green (1943) and the film and direct-spray techniques of Tattersfield & Potter (1943). The same species of test insect, *Tribolium castaneum* is used in all

four methods. The numbers of badly affected + moribund + dead insects were counted by Tattersfield & Potter, whereas in the other two techniques dead insects only are counted. Tattersfield & Potter exposed beetles on films formed on tricoline, and confined beetles on the tricoline circles on which the beetles had previously been sprayed. As the ranges of concentration and deposit in their film tests overlapped those in their direct spray tests, there must have been some film effect in the latter. This conclusion is further supported by the fact that probit planes obtained by the two methods did not differ significantly in position.

In the direct-spray method described in this paper film effects are reduced to the minimum practicable. They are confined to the contact of the insects with sprayed surfaces during spraying, and the contact of the sprayed insects with each other as they are swept into containers for storage, if the latter can be called a film effect. Film effects between spraying and observation of results are avoided by the transfer of the sprayed insects to clean filter-papers. Further reduction of film effects might be possible, but the consequent elaboration of the technique would probably reduce unduly the numbers of insects that could be sprayed in one day.

In both the film technique of Parkin & Green (1943) and the direct-spray technique described in this paper, the data fit regression lines within the limits of sampling, as shown by χ^2 tests, so in this respect the two techniques are equally satisfactory. The regression coefficients given by these two techniques were as follows:

	Regression coefficients	
Technique	Log-concentration	Log-deposit
Film	3·5	12
Direct spray	8·0	3·8

The coefficients for the film technique were not taken from Parkin & Green (1943), but were obtained by the slightly modified version of their method (see p. 358) using the same stock of *T. castaneum* as was used in the experimental work described in this paper. In the film technique the coefficient of regression on log-deposit is greater than that on log-concentration, and in the direct spray technique vice versa. Other things being equal, the higher the coefficient of regression on log-concentration, the closer together will the limiting and standard concentrations be. On this basis the direct-spray technique is slightly better than Parkin & Green's film technique; but both methods are valuable in bio-assay work with suitable insecticides, and should be regarded as complementary.

This work has formed part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research. I am indebted to Mr R. L. Plackett, of the Mathematics Division, National Physical Laboratory, for developing equation (1). Miss B. Clayton gave considerable assistance in the experimental work and in the computation of the regression lines.

Fig. 2*a*

Fig. 2*b*

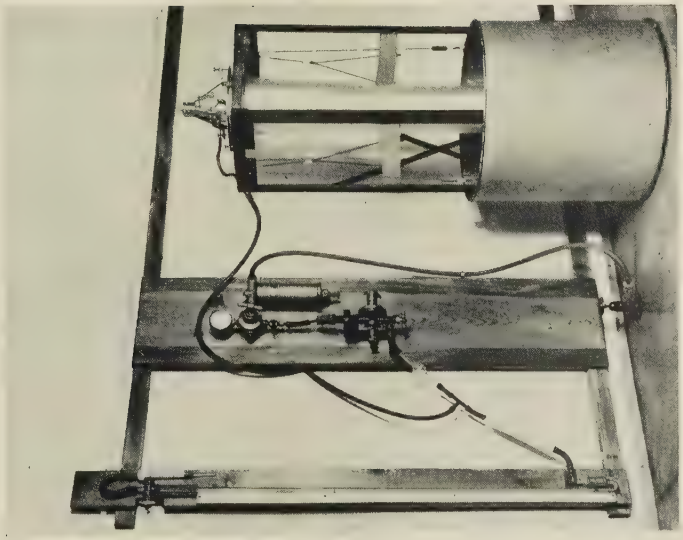


Fig. 1

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EXPLANATION OF PLATE 7

Fig. 1. Modified form of the spraying tower designed by Potter (1941).

Fig. 2. Apparatus for confining insects during spraying: (*a*) showing separate parts, left to right; duralumin plate resting on filter-paper covering cardboard disk, filter-paper, duralumin ring, brass mask; (*b*) set up and containing insects.

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THE RELATION BETWEEN THE SIZE OF PLANT AND THE SPREAD OF SYSTEMIC DISEASES

I. A DISCUSSION OF IDEAL CASES AND A NEW APPROACH TO PROBLEMS OF CONTROL

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It is implicit in the concept of systemic disease that there should be a connexion between the size of the plant and the spread of infection.

A first, general theorem, thought to be universally valid, is: The spread of an infectious systemic disease increases with the size of the plants, size being appropriately determined and other factors being constant.

A second theorem for infections which enter a crop from without (e.g. from the soil or from another field) is: When a systemic disease enters a crop from some uniform outside source, the logarithm of the proportion of healthy plants is directly proportional to the size of the plants, other factors being constant; as an approximation, the proportion of infected plants is directly proportional to their size, provided that the proportion is small. A corollary to this theorem is that if the proportion of infected plants is small and if the size is inversely proportional to the density of planting, the number of infected plants per unit area will be approximately constant, factors other than size and number being constant.

A third theorem for the spread of infection within a crop is: When a systemic disease spreads within a crop, the rate of infection is proportional to the size of the healthy plants, other factors being constant. If factors other than size, time and the proportion of infected plants are constant, the expression for the rate takes one of the three forms:

$$\frac{dx}{dt} = ks\,(1-x), \tag{I}$$

$$\frac{dx}{dt} = ks\,(1-x)\,x, \tag{II}$$

or

$$\frac{dx}{dt} = ks\,(1-x)\,f(s, t, x), \tag{III}$$

in which x and $1-x$ are, respectively, the proportions of infected and healthy plants, s is the size, and k is a constant. The second part of this theorem is tentative.

In these theorems we take by definition a disease to be systemic if a single effective transmission suffices to bring about maximum infection of the plant. Size refers to that part of the plant which can receive infection. This definition, with the size of the receiving part having its colloquial meaning, serves for the first, general theorem. For quantitative relations, the guise but not the substance of the definition is changed: size is proportional to the probability that the plant receives infection at any instant from a uniform source in appropriate circumstances. Both for general and quantitative relations there is a special case when disease is introduced *into* a crop by insects or other vectors. Size, then, is the catchment zone of the plant for the insects. This accords with the meaning assigned to size in other circumstances; size is here a convenient fiction, the use of which gives precisely the same results as the use of numbers, which are a reality.

Reduction of size is discussed as a measure of control peculiar to systemic diseases.

Introduction

A systemic plant disease is one which extends through the whole susceptible portion of the plant, whatever its size may be. With any single variety of plant and any single systemic disease in any specified set of conditions, the larger the plants, the greater is the multiplication of disease following a single effective transmission, the greater the newly developed source of infection for transmission to other plants, and the greater the likelihood of further transmission. The connexion between the development of infection and the size of the plant is implicit in the concept of systemic disease. One need not demonstrate it experimentally; it is axiomatic. What concerns us is to formulate the consequences quantitatively. This will be done by first considering certain uncomplicated, 'ideal' cases, just as in studying the properties of gases it is convenient to begin by studying 'ideal' gases, and leave complications for probing later.

It is simplest to divide the problem, and consider, first, cases of infection reaching the crop from an outside source and, secondly, the spread of infection within a crop. In practice, one is commonly concerned with a combination of both aspects of the problem, and the disease in a field is often the joint result of infection arriving from without and accumulating within. For present purposes, however, these combinations are unimportant. If each aspect can effectively be dealt with separately, dealing with a combination is largely a matter of formulation.

Before proceeding to treat the subject quantitatively, we may usefully consider in some detail a general underlying theorem which anticipates two particular theorems and which is thought to be universally valid. It is:

The spread of an infectious systemic disease increases with the size of the plants, size being appropriately determined and other factors being constant.

For convenience, this will be called the first, or general theorem.

Dictionaries define systemic diseases as diseases of the system as a whole. The definition is not altogether tenable. Most viruses do not enter the true seed of plants; the virus of raspberry leaf-curl or of sugar beet curly-top is more or less limited to the phloem (Bennett, 1927, 1934); the virus of streak disease of maize is difficult to recover from green areas (Storey, 1928); and other familiar examples serve to show that most infections commonly accepted as systemic do not extend through every part of the plant. A better definition, with which all expressions in this paper conform, is that a disease is systemic if a single effective transmission suffices to bring about maximum infection of the plant. The meaning of 'effective transmission' is clear: a sufficient mass of infection must reach a part of the plant where the infection can, and actually does, multiply; a virus spreading through the phloem must be injected and established in the phloem, and a vascular fungus must reach and spread in the vascular system.

To avoid complicated enunciation, the word 'plant' is used. In special circumstances it must be taken to include parts of a plant. For example, many of the rots of

stored fruits and vegetables are systemic diseases. It might be added that the word is not intended to be exclusive; the implication that the spread of systemic diseases varies with size holds for the animal kingdom as well, but that does not concern us here.

Size refers to the part which receives infection. If a disease is transmitted by an active insect to the leaves only, it is the foliage that counts. If a disease is spread by contact between leaves, it is those leaves of a plant which are in contact with other plants that count. No other part is taken into reckoning. If, for example, the disease enters a healthy plant only through the leaves, one does not reckon in the roots or tubers, even though infection travels there systemically and even though, as sometimes happens (e.g. with the potato viruses), the roots or tubers of diseased plants sooner or later serve indirectly as the source of infection for the leaves of healthy plants. A considerable degree of precision is desirable—if, for example, a systemic disease enters only at the root hairs, none of the rest of the root zone counts—and the greater the precision, the better the first, general theorem will hold.

This definition, with size used in its colloquial sense but related only to the part receiving infection,* suffices for the general theorem. For the quantitative theorems which follow, it is convenient to change the form of the definition; and to get matters of definition over at once, this change can profitably be discussed now. For the quantitative theorems, the size of a plant is taken to be proportional to the probability that infection will be received by the plant at any instant from a uniform source in appropriate circumstances. The substance of the original definition remains. One may illustrate this by an uncomplicated example which will be familiar to anyone who has dug potato plants. Picture two seed-potatoes, the one producing a single sprout which branches into two before emerging from the soil, the other producing two sprouts from different eyes which emerge unbranched. From above, the 'plants' which grow will look alike; but, in the first case, there will be only a single plant, while, in the second, the 'plant' will be in reality two plants, as soon as the seed rots. If, at any instant, an aphis injects virus, it will, in the first case, ultimately infect the whole plant; in the second, one of the two plants. The probability of the plant becoming infected in the first case is twice that of *either* of the plants in the second case. In the first case, the plant would be twice the size, as defined by reference to probability, of either in the second case; it is also twice the size in the colloquial meaning of the word.

If, to continue the illustration, the plants were exposed to infection over a long period, there would be a possibility of repeated transmissions of virus. The simple relation between size and the probability of infection would then disappear. This is the reason why the definition limits the probability of infection to an instant of time.

* There are plenty of precedents for a restriction of this sort. For example, 'force' in physics has a more restricted meaning than in colloquial English, but physicists do not deplore its use on that score.

The general intention of these definitions, both for the general theorem and for the quantitative theorems, is clear, even if the guise is not always familiar. There is, however, one particular case, that of disease brought *into* a crop by insects or other vectors, which is less straightforward. Consider, for example, a field of young plants newly emerged from the soil. Vectors alighting in the field will make for a plant (assuming the field is free from weeds which are favoured hosts of the vector), and however small the plants themselves may be, they will receive an infestation which is proportional to the mean area of ground around them. Size, in this case, will be the catchment zone of the plant, and not the size of the plant itself. This definition conforms with the principle, adopted throughout, that size is a measure of how the disease is received, or, in its quantitative guise, that size is proportional to the probability that infection be received at any instant. The objection that size is now a fiction is discussed later, when it will be shown that precisely the same conclusions are reached if, instead of dealing with fictional sizes, one deals with real numbers. For the present we shall consider that size is *always* the catchment zone for the invading insects; and is *usually* inversely proportional to the number of plants per unit area, some exceptions being when plants are separated by distances greater than those over which they can attract the insects, or when weeds, attractive to the insects, are present.

The qualification that other conditions be constant needs no explanation; and it is to be understood throughout this paper that size is considered as an isolated and independent factor.

Cases in which infection reaches the crop from an outside source

Consider infection brought into a crop from some uniform source without—from the soil, uniformly infected with a virus disease like big-vein disease of lettuce or a bacterial and fungus disease* which attacks systemically, or from another crop or other plants. If x_1 is the proportion of infected plants of size s_1, the proportion of healthy plants—the proportion of plants receiving no infection at all—is

$$1 - x_1 = e^{-m_1},$$

where m_1 is the mean number of transmission to each plant. This relation is given by the Poisson theorem.

Similarly, if x_2 and m_2 are the corresponding quantities for plants of size s_2,

$$1 - x_2 = e^{-m_2}.$$

Whence

$$\frac{\log (1 - x_1)}{\log (1 - x_2)} = \frac{m_1}{m_2}.$$

* As a general rule, bacterial and fungus diseases have not as great a tendency as virus diseases to spread systemically; and even parasites like *Xanthomonas solanacearum* which normally spread quickly through a plant can be localized if conditions are unfavourable to them. But it is no purpose of this paper to discuss the conditions which permit systemic development, or to draw up a list of systemic diseases; our concern here is purely with the consequences of the fact of systemic development.

From the definition of size as proportional to the probability of an infection being received at any instant from a uniform source, it follows that the number of transmissions over a given period of time is directly proportional to size, other conditions being constant; that is,

$$\frac{m_1}{m_2} = \frac{s_1}{s_2}$$

and

$$\frac{\log (1-x_1)}{\log (1-x_2)} = \frac{s_1}{s_2}. \qquad (1)$$

This is a general relation, applicable to all proportions of infected plants. If the proportion is low, one may simplify matters by using an algebraic transformation and, *as an approximation*, write

$$\frac{x_1}{x_2} = \frac{s_1}{s_2}. \qquad (2)$$

The smaller the proportion, the better is the approximation. When x_1 and x_2 are less than 0·1 the simplified equation may be considered accurate enough for most purposes; when less than 0·2, useful as a general approximation.

One may collect equations 1 and 2 in a general theorem:

When a systemic disease enters a crop from some uniform outside source, the logarithm of the proportion of healthy plants is directly proportional to the size of the plants, other factors being constant; as an approximation, the proportion of infected plants is directly proportional to their size, provided that the proportion is small.

As examples of calculations from equation 1, halving the size of the plants will reduce infection (other things being constant) from 99 to 90%; from 50 to 29·3%; from 20 to 10·6%; from 10 to 5·14%; and from 2 to 1·005%. These figures also illustrate the nature of the approximation inherent in equation 2.

One might interpolate here that if infection is introduced by insects (or similar agency), equations 1 and 2 should hold even if the insect does not stop at the first plant it feeds on, provided that it moves about randomly. If the insects arrive without virus, pick up infection within the field, and then move about, the case will not be one of infection arriving from without, and will be left to the section dealing with spread within a crop.

The special case in which size is inversely proportional to the number of plants per unit area. Consider plots of unit area exposed to a uniform source of systemic infection, such as uniformly infected soil, or a uniform population of migrating vectors. Suppose the total number of plants (healthy and infected) per plot—that is, the density of planting—varies, and that the size of the plant is inversely proportional to the number. If $n_1, n_2, \ldots, n_n, \ldots$ is the total number of plants, $\mu_1, \mu_2, \ldots, \mu_n, \ldots$ the number of infected plants, and $x_1, x_2, \ldots, x_n, \ldots$ the proportion of plants infected in plots 1, 2, ..., n, ..., $x_1, x_2, \ldots, x_n, \ldots$ being assumed to be low; then, adapting equation (2) by introducing numbers inversely proportional to sizes,

$$n_1 x_1 = n_2 x_2 \ldots = n_n x_n \ldots \quad \text{or} \quad \mu_1 = \mu_2 \ldots = \mu_n \ldots \qquad (3)$$

This result may be incorporated as a corollary to the second theorem:

If disease from some uniform outside source attacks a small proportion of the crop, and if the size is inversely proportional to the density of planting, the number of infected plants per unit area will be approximately constant.

Factors other than size and number are presumed constant.*

It might seem that this corollary is hedged in with qualifications, particularly that size is inversely proportional to number, and that the proportion of infection must be low (so that equation 2 can apply). But the qualifications are met more often than might appear at first glance. When infection is introduced by migrating insects, size has been defined as the catchment zone of the plant and, unless plants are separated by distances greater than those over which they can attract the insect, is usually automatically inversely proportional to the number per unit area. In other cases, when plants are spaced densely enough to compete strongly with one another, size is conditioned by number and often tends to become inversely proportional to it.

The spread of systemic disease within a crop

A third theorem seems to cover all cases. It is:

When a systemic disease spreads within a crop, the rate of infection is proportional to the size of the healthy plants, other factors being constant. If factors other than size, time, and the proportion of infected plants are constant, the expression for the rate takes one of three forms:

$$\frac{dx}{dt} = ks\,(1-x), \tag{I}$$

$$\frac{dx}{dt} = ks\,(1-x)\,x, \tag{II}$$

or

$$\frac{dx}{dt} = ks\,(1-x)\,f\,(s,\,t,\,x), \tag{III}$$

in which x and $1-x$ are, respectively, the proportions of infected and healthy plants, s is the size, and k is a constant.

That the probability of any particular plant becoming infected at any instant of time is proportional to its size, is contained directly in the definition of size. It is evident, too, that the rate at which plants become infected is proportional to $1-x$, when the disease is dispersed randomly, for example, by active insects, so that all the healthy plants stand in equal danger of infection. But when, for example, the disease spreads by contact, so that healthy plants out of contact with diseased plants are not in immediate danger, the connexion is less evident, and is best demonstrated informally by discussing this particular example.

Suppose the limitation in spread is extreme, and that the disease passes only between immediate neighbours in a row. To ascertain how infection will build up,

* The writer is indebted to Mr F. C. Bawden for the information that from field experiments with difference virus diseases at Rothamsted he had arrived at the tentative conclusion that the number of plants infected was a constant per unit area rather than a percentage of total plants.

it is necessary to know how diseased plants are grouped, because a run of diseased plants will be no more capable of spreading disease than a single healthy plant; the only danger will be to the healthy plants on each side of the run or of the diseased plant, as the case may be. A convenient procedure is to determine the number of 'doublets'. A doublet is any pair of adjacent diseased plants, a run of three diseased plants being two doublets, a run of four, three doublets, and so on. If there are n plants in a row and, of these, μ are diseased, the expected number of doublets is $\frac{1}{n}\mu(\mu-1)$ (van der Plank, 1946). The number of direct contacts between healthy and diseased plants is thus $2\left[\mu-\frac{1}{n}\mu(\mu-1)\right]$ or, in long rows, simply $2\left(\mu-\frac{\mu^2}{n}\right)$. In making up this figure, a healthy plant between two diseased is counted as two contacts. The rate at which plants become infected is proportional to their size (as defined) and the number of contact surfaces; that is, assuming that all infected plants are infective,

$$\frac{n\,dx}{dt} \propto 2s\left(\mu-\frac{\mu^2}{n}\right), \quad \text{or} \quad \frac{n\,dx}{dt} = ks\left(\mu-\frac{\mu^2}{n}\right),$$

where k is a constant in which is included the factor 2. Rearrangement gives

$$\frac{dx}{dt} = ks\left(1-\frac{\mu}{n}\right)\frac{\mu}{n}.$$

Since

$$\frac{\mu}{n} = x, \quad \frac{dx}{dt} = ks\,(1-x)\,x.$$

Despite the fact that at any moment only part of the healthy plants are in danger, the term $1-x$ remains, and the equation takes precisely the same form as it would if the disease were scattered randomly by some far-flying, free-breeding insect.

A formal proof for all intermediate cases between the extremes of random scattering and contact spreading is desirable, and also proof for equations of form III. Until this is available, our conclusions must be considered tentative.

To turn to the form of the equations, form I,

$$\frac{dx}{dt} = ks\,(1-x),$$

occurs normally when the time over which infection develops is less than the time needed for infected plants to become infective. This circumstance is probably less rare than might be supposed at first thought. For example, in many climates a heavy aphis infestation of potatoes is of short duration; numbers rise to a peak and fall again, and if the peak is sharp enough, the time may be insufficient for the newly infected plants to pass infection on. Early varieties, too, may be lifted soon after infestation has reached an appreciable degree, and before new infections can be passed on. The sole source of infection in these examples is the plants infected at the beginning of the season, and their proportion is included as a factor of k.

A simple derivation of form II,

$$\frac{dx}{dt} = ks\,(1-x)\,x,$$

has already been given. In all probability, equations of this form are only approximations, because they assume that all infected plants are infective, but they are useful simplifications when the spread of infection is considered over long periods.

Form III is probably the most common.

Often equations take the form

$$\frac{dx}{dt} = ks\,(1-x)\,x_{t-P},$$

in which x_{t-P} is the value of x at time $t-P$. P represents the period needed for infected plants to become infective, or the incubation period of a virus in an insect, or the sum of these two periods. The same equation can also be used for transmissions like those of the tomato spotted wilt virus by thrips. The adults transmit the disease only if it was acquired in the larval stage, and the number of infective adults in a field depends on the proportion of infected plants at the time they were larvae. To integrate this equation, x_{t-P} must be replaced by some function of s, t and x, most conveniently and fairly accurately by the term $x-P\frac{dx}{dt}$.

Form III is also appropriate when infected plants differ in size from healthy plants. If all infected plants were the same size, this difference would not matter, but ordinarily the relative size of an infected plant will depend on how long it has been infected.

All three equations are concerned only with the proportion of infected plants, size and time. Other conditions—the environmental conditions in which the plants grow, for example—are assumed constant, the constant k being the expression of this assumption. Ordinarily, the achievement of constancy in environment and other factors is impossible. Nevertheless, it is probably possible to isolate and exclude to a large extent the effects of changing factors. For example, one might study the effect of size by growing plants of different sizes in the same environment over a definite season or period of time; or, with diseases which take years to spread through a crop, one may make the year (or the season) the unit of time, and assign to k a value which is the mean for a year.

Discussion

The relation between size and number. Objection may be taken to the definition of size as the catchment zone, when infection is brought into a crop by insects or other vectors. We grant the validity of the objection. At its best, the definition is a fiction which was used to bring about uniformity of treatment, and to discuss the problem in terms of size throughout. In reality, the problem is one of number: the spread of the insects over a number of plants.

But if size is a fiction in this case, it is at least an accurate fiction, and by adopting it one reaches precisely the same result as by working with numbers. Let us go back to equations 1 and 2, and repeat the arguments using numbers instead of sizes.

Consider two fields exposed to a uniform environment of migrating infective insects. If m_1 and m_2 are the mean number of transmissions of infection per plant in the two fields, and x_1 and x_2 the proportions of disease developed,

$$1-x_1=e^{-m_1}, \quad \text{and} \quad 1-x_2=e^{-m_2}.$$

Thus

$$\frac{\log(1-x_1)}{\log(1-x_2)}=\frac{m_1}{m_2}.$$

The mean number of transmissions per plant can reasonably be assumed to be proportional to the number of vectors per plant, and, since the vector environment of the two fields is uniform, this is inversely proportional to the number of plants per unit area. Thus

$$\frac{m_1}{m_2}=\frac{n_2}{n_1}, \quad \text{and} \quad \frac{\log(1-x_1)}{\log(1-x_2)}=\frac{n_2}{n_1}, \tag{1A}$$

in which n_1 and n_2 are the number of plants per unit area in the two fields.

When x_1 and x_2 are small, one may write as an approximation

$$\frac{x_1}{x_2}=\frac{n_2}{n_1}. \tag{2A}$$

From this, equation 3 may be derived directly.

Equations 1A and 2A are the counterparts of equations 1 and 2, when number is used instead of size. In numerical calculations they give exactly the same results. A condition for their use is that the distance between plants must be smaller than the distance over which the plants attract the insects. If this condition does not hold—if the distance between plants is greater—one cannot use equations 1A and 2A, that is, the equations which use numbers break down. These equations also break down when weeds, attractive to the vector, are present. But equations 1 and 2, which use size, are still valid, size, defined as the catchment zone of the plant, in this case not being inversely proportional to number. Size, however much a fiction it may be, has some advantage over number. The use of number, however real, is not always legitimate, and, moreover, requires separate treatment for those systemic diseases which are carried by invading insects (or similar agents). The use of size, even if it is fictional at times, permits uniform treatment of all systemic diseases on all occasions. In the circumstances we prefer to use size, but do not wish to be dogmatic; when dealing with diseases brought by insects or other vectors into a field clear of weeds and with normal spacing of plants, it is not only permissible but sensible to simplify matters by using numbers.

The value of 'ideal' cases. All the theorems contain the condition that other factors should be constant. This condition commonly does not hold. Nevertheless, there is great value in 'ideal' cases. This one may show with the virus of tomato spotted wilt, which also attacks tobacco and pine-apples.

Under South African conditions the vector rarely moves from the tobacco leaf on which it settles. Coming into fields of Virginia-type tobacco without flowers, the vectors settle and infect plants randomly. They apparently do not multiply in the fields; infection all comes from without and does not spread within the field. The experimental data fit the second theorem closely, as the results of van der Plank & Anderssen (1945) show. Similar figures were obtained with pine-apples in Hawaii by Linford (1943), who realized the importance of number but not the mathematical complexity of the problem. But with tomatoes in South Africa the incidence of disease is decreased by decreasing size, but not to the extent demanded by the second theorem, even when it is certain that all infection has come from without. The writer's own suggestion, which is yet not more than an unproved guess, is that the tomato is so much to the liking of the vectors that they are attracted in greater numbers to plots with bigger, or more closely spaced, plants. In other words, it is suggested that other factors are not constant, and that plots are exposed to an environment of vectors which is not uniform but varies with the mass of foliage or flowers per unit area of plot. Tobacco and pine-apples, on the other hand, are not specially favoured hosts, and there is good evidence that they do not attract the vectors over any appreciable distance. Whether this suggestion about tomatoes is correct or not, is not very important for our present purpose. What is important, is that there is a gain of information, whether the case deviates from the 'ideal' or not. Because the 'ideal' is followed with tobacco and pine-apples, one learns much about the disease; because it is not followed with tomatoes, one also learns something about the disease and can think of new lines to follow. Without an 'ideal' case for comparison, the experimental evidence would be a tangled mass of unexplained details with all three hosts.

As a general rule it would seem that 'ideal' cases have most value when one deals with strong parasites. Weak parasites, like those moulds of fruit which attack only through an injury or weakened skin, are necessarily sensitive to the environmental and other conditions; and, with them, the condition, that factors other than size should be constant, looms overlarge in the theorems.

The reduction of size as a measure of control. Numerical calculations show that systemic disease is hardest to control by reducing size, when the percentage of infection is high. The reduction of infection is then far from proportional to the reduction in size. Control is however possible with high percentages of infection in the particular case of a systemic disease which strikes when the plants are small. One may then plant thickly, and keep a large population until the plants begin to crowd one another and need thinning. In this way one is concerned not so much with the plants which become infected, but with those which survive. This method works well with spotted wilt of tobacco in South Africa, which generally strikes early in the season, and a table has been constructed to show the benefit of planting two and three plants per hill (van der Plank & Anderssen, 1945).

For systemic diseases which attack when plants are mature, it is a condition for

control that the percentage of disease must be reasonably low. The great advantage of the method is that it applies to all systemic diseases; when a crop is subject to many of them, as tomatoes are to various systemic virus, fungus and bacterial diseases, one has a measure of insurance against all of them, each independently of the other. In South Africa experiments are under way to ascertain the effect of replacing tomato plants with three or four main branches by three or four plants each pruned to a single stem. By doing this one can multiply the number of plants per unit area without greatly affecting the mass of plants and all that goes with it. Only the size is changed. Another project, to give a second example to illustrate the flexibility of the method, is to raise nucellar (apomictic) citrus seedlings in an attempt to throw off any viruses which may be present without losing the advantages of vegetative propagation, bud these on to dwarfing (*Poncirus trifoliata*) stock, and plant out at the rate of thousands to the acre or maintain as dwarfs in pots quarantined in a glasshouse. These plants would then provide buds for ordinary stocks to be planted at ordinary spacings.

This brings us to a point about the problem of maintaining stocks of vegetatively reproduced crops in health. Crane (1945) has suggested *inter alia* that some of the dangers might be avoided by the regular production of short-lived clones. One assumes here that the clones would last for a few years at least, in which case it can be asserted with fair assurance that the time taken for infection to increase from any one level to another would be inversely proportional to the size of the plants;* that is, the value of Crane's suggestion can be made much greater when it is possible to keep parent stocks small by some means or other. This often requires keeping a parent stock for no purpose other than propagation, but this would be worth while.

There is another way of viewing the control of systemic disease by reducing size. Shorn of all mathematical consideration, it is a step in the same direction as controlling disease by localizing the lesions. If one can confine infection to a local lesion, as the virus of tobacco mosaic is localized in *Nicotiana glutinosa*, the disease is for all practical purposes controlled. Failing localization of this sort, the next best

* If the clones last a few years at least, the time taken for infection to build up is usually, but not always, much longer than the time needed for infected plants to become infective, and one may use an equation of form II in the third theorem. If a is the proportion of infection at the start, that is, if it is the lower level from which infection develops, one may modify the equation and write:

$$\frac{dx}{dt} = ks\,(1-a-x)\,(a+x),$$

in which x is the proportion of infection developed thereafter in time t. By integration,

$$t = \frac{1}{ks}\log\frac{(1-a)\,(a+x)}{a\,(1-a-x)}.$$

Here t is measured in years and k, a constant, has the mean value for the year. If t_1 and t_2 are the times needed to reach any particular level of infection when the sizes are s_1 and s_2,

$$\frac{t_1}{t_2} = \frac{s_1}{s_2}.$$

Slight departures from this 'ideal' equation would not appreciably affect its practical significance.

step in the same line is to confine the disease as much as possible by limiting the size of the plant. In both methods the underlying principle is that the sting of a systemic disease is in its ability to extend through a plant after a single successful transmission, and that anything is necessarily good which confines it, at least to those parts which receive infection or which, directly or indirectly, transmit it.

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VIRUS DISEASES OF CACAO IN WEST AFRICA

I. CACAO VIRUSES 1A, 1B, 1C AND 1D

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(With Plates 8 and 9 and 1 Text-figure)

As an introduction to this series of papers, a brief history is given of swollen-shoot disease of cacao in the Gold Coast, and the nomenclature for cacao viruses is discussed.

The symptoms of four virus diseases of cacao are described in detail, together with experimental data under the headings: cross-immunity reactions, latent period, seed transmission, staining reaction, heat inactivation and rate of spread.

Introduction

Virus diseases of cacao (*Theobroma cacao* L.) have attracted general attention recently because of their serious effect on the production of cacao on the Gold Coast, where swollen-shoot disease is a menace to the economic stability of the colony, cacao beans being the primary agricultural export. The cacao viruses are also of scientific interest as they present a number of unusual or even unique problems.

The development of the Cocoa Research Station of the Gold Coast Department of Agriculture into The West African Cacao Research Institute in 1944, with concomitant increases in staff and finance, made possible intensive research on cacao viruses. A series of papers, of which this is the first, will be published by research workers at W.A.C.R.I. as suitable stages of their investigations are reached.

In 1936, the attention of W. F. Steven (Steven, 1936) was drawn to swellings on some cacao branches brought to the Department of Agriculture by an African farmer in the New Juaben district of the Eastern Province of the Gold Coast. On investigation, Steven found that these swellings were associated with a severe disease which he named 'swollen-shoot and die-back'. An eradication campaign was commenced immediately by the Department of Agriculture but was abandoned when the disease was found to be widely distributed. After the efficacy of rogueing was demonstrated at Tafo (Posnette, 1943*c*) a new campaign was started in 1941 and met with considerable success (Anon. 1945).

The most heavily infected area lies between Akwadum and Nankese and recent investigations (Paine, 1945) have traced the history of dying cacao, almost certainly due to swollen-shoot, near Nankese back to 1922. Swollen-shoot has devastated cacao farms in the Eastern Province, which was the most intensive cacao-producing area in the world until surpassed by Ashanti in 1942; production has decreased from 116,000 tons in 1936–7 to 64,000 in 1945–6. Not all of this decline has been caused

by swollen-shoot, as capsid pests, drought and soil deterioration have contributed, but £2 million is a conservative estimate of the capital loss in trees killed by the virus up to 1945 (Anon. 1945). The annual loss is, of course, progressive but at the 1945 price of cacao is probably not less than £1 million. (The price paid to the Gold Coast producer for cacao beans in 1946–7 was twice that paid in 1945.)

Virus diseases are now known to occur in all the main cacao-growing areas of the Gold Coast, as well as in the Ivory Coast and Nigeria. Except in the Eastern Province of the Gold Coast, the outbreaks are not very extensive and symptoms often differ with locality. These symptom-differences have been reproduced constantly in graft and insect transmissions to seedlings under uniform conditions, and there is no doubt that they represent different viruses or strains of a virus complex. All seedlings used for transmission experiments are of the West African Amelonado type which shows a high degree of genetical uniformity. Symptom variation between seedlings infected with the same virus has been no greater than between plants of the same clone. Between clones of the Trinitario type, however, there is considerable variation in the symptoms induced by a virus strain. Baker & Dale (1947), describing virus diseases of cacao in Trinidad, have preferred the names 'Strain A' and 'Strain B' to 'red-mottle' and 'vein-clearing' because these symptom differences are not constant on all clones. This objection would apply to the name 'swollen-shoot', and indeed to many descriptive names commonly applied to viruses.

No attempt was made to distinguish between the symptoms caused by different viruses or different strains in the writer's previous descriptions of swollen-shoot (Posnette, 1940, 1943*a*), although it was known in 1940 that there was more than one (Posnette, 1941). Now that more than ten variants have been distinguished, the diagnostic symptoms of each will be described. The following paper deals only with the first four to be recognized.

Cacao viruses 1 A, 1 B, 1 C and 1 D

Nomenclature

The name 'swollen-shoot' has been adopted by the Imperial Mycological Institute (Wiltshire, 1946) for the disease recognized by Steven and for the virus first graft-transmitted by the writer (Posnette, 1940). The question of the relationship of other cacao viruses to swollen-shoot is an open one. In the absence of serological tests, cross-immunity provides the only test for affinity and there is some evidence with bacterial viruses (Delbrück, 1946) that serological and cross-immunity reactions do not always agree. The first paper deals with four viruses which for simplicity will be termed strains A, B, C and D without prejudice to their possible relationship as distinct viruses or strains of the swollen-shoot virus.

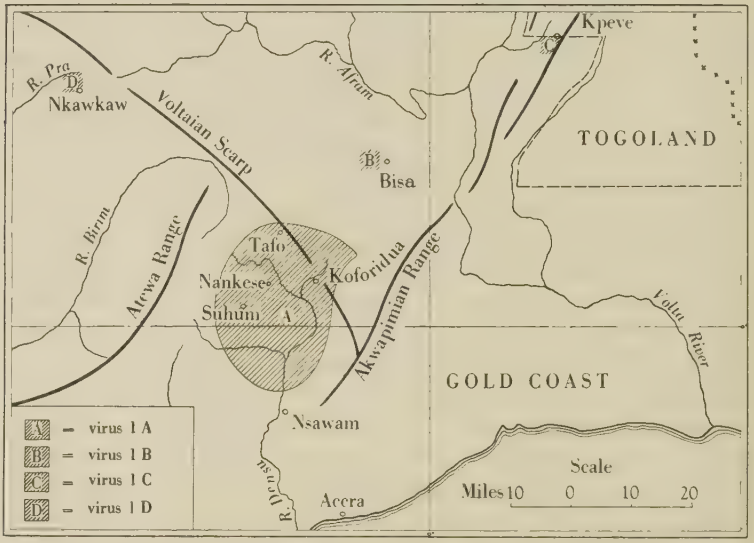
A list of letters in use for cacao virus strains was distributed in 1944 (Posnette, 1944) and adopted by Alibert (Alibert, 1946). Letters have been used in all W.A.C.R.I. reports since 1944, and in a review (Posnette, 1945*a*) dated February, 1945; they therefore take precedence over the numbers used by Box (Box, 1945) for

the same strains (supplied by the writer) and it seems preferable to use letters for strains, reserving numbers for distinct viruses.

Under Smith's classification (Smith, 1937), swollen-shoot strain A should be termed *Theobroma* virus 1A. According to Holmes' terminology it would be *Marmor theobromae* var. A.

Distribution

Text-fig. 1 shows the localities in which these four viruses are known to occur; however, our knowledge is probably incomplete as regards strains B and D which do not cause any obvious defoliation. Strain A, the most virulent, is the most widely distributed; its greatest concentration is in the New Juaben district, where most of



Text-fig. 1. Distribution of main outbreaks of cacao viruses 1A, 1B, 1C and 1D.

the remaining cacao farms are in various stages of infection, but the infected area covers most of the cacao-growing land of the Eastern Province. Strain B was originally recorded from the Bisa district, where so much cacao has since been killed by unsuitable edaphic conditions and by other virus strains that it is impossible to estimate the extent of B infection. Small outbreaks of strain B have been found also in the New Juaben district.

Strain C is known from only one outbreak isolated from other strains and covering about 5 acres on the Gold Coast-British Togoland border. As the symptoms of C are conspicuous, it is probable that this is the only place where it occurs.

Outbreaks of strain D have been found only near Nkawkaw, a district with scattered A infections.

Description of strain A

Common name	Swollen-shoot strain A.
Scientific names	*Theobroma* virus 1A, *Marmor theobromae* var. *A.*
Synonyms	New Juaben strain; virulent strain.
Type locality	Effiduase, New Juaben district, Gold Coast (1936).
Vectors	*Pseudococcus njalensis* Laing (Cotterell, 1943, Box, 1945); *Ferrisia virgata* Ckll. (ibid); *Pseudococcus citri* Risso (Posnette, unpublished).

Symptoms

Swellings. Swellings are produced on the fan and chupon* branches and on the tap and lateral roots. Swellings are sometimes pronounced on suckers arising from the base of the trunk, amounting to twice the diameter of the unswollen stem; on fan branches the swellings are slight. Swellings may be nodal or internodal, but owing perhaps to the severe check in growth caused by this virus they are often terminal and associated with the death of the apical bud (Pl. 8, fig. 1).

The swellings are caused almost entirely by a localized increase in xylem tissues, vessels being larger and more numerous and xylem parenchyma broader in transverse section. Only slight proliferation of phloem tissue occurs. (For anatomical drawings of swellings, see Mangenot, Alibert & Basset, 1946.)

The interval between time of inoculation and the formation of swellings is very variable; it may be as short as 2 months or occasionally a plant may be killed by the virus before obvious swellings develop.

Leaf symptoms. The leaf symptoms of strain A, resembling those of other virus mosaics, can be classified according to their anatomy into the following types:

(1) Vein-clearing, usually of the finest macroscopic (fourth-order) veins, accompanied by slight reduction of the chlorophyll in the adjacent mesophyll (Pl. 8, fig. 2).

(2) Vein-banding of tertiary or fourth-order veins, actually caused by the clearing of the microscopic veins adjacent to those apparently banded (Pl. 8, fig. 3).

(3) Opaque yellow or yellowish green areas in which there may be some reduction in palisade tissue, but differentiation is usually complete and the yellow colour is due to the chloroplasts being smaller, less numerous and with slighter development of chlorophyll than in the unaffected part of the leaf.

(4) Transparent pale yellow or white lesions containing little or no differentiation of the mesophyll, the lamina being thinner owing to the absence of palisade tissues and air spaces. Chloroplasts are few and small. These lesions may become necrotic as the leaf matures.

There is considerable variation in the pattern of the chlorotic areas forming mosaic as the plant passes from the acute to the chronic phase of infection. Leaves of the cacao plant are produced in periodic growth phases (flushes) divided by

* *Chupon*: shoot with $\frac{3}{8}$ phyllotaxis usually arising from main stem. *Fan*: secondary shoot with $\frac{1}{2}$ phyllotaxis (see Cheeseman, 1934).

dormant phases (Humphries, 1944); symptoms appear only on leaves formed after infection. If the interval between infection and first symptoms is short (usually less than 1 month), only a clearing of some of the fourth-order and microscopic veins occurs, producing either a fine yellow network or a few scattered flecks (Pl. 8, fig. 2). The next flush of leaves then shows what is commonly the first symptom when the latent period is more than 1 month—a fine-grain mosaic over the whole lamina. Mosaic varies greatly in intensity, but is usually composed of pale yellow polygons, delimited by the finest macroscopic veinlets, on a 'background' of normal green (Pl. 8, fig. 4). In the most intense form, the yellow (type 3) lesions coalesce into blocks between the main veins and transparent (type 4) lesions may be superimposed (Pl. 8, fig. 5). This is often accompanied by downward curling of the leaf edges and inter-veinal crinkling. Necrosis of the leaf tip and of the transparent lesions frequently occurs in this acute phase.

This mosaic pattern may be reproduced in several successive flushes, but is gradually restricted to the sides of larger veins until in the chronic phase it is confined to the secondary and short lengths of the tertiary veins as a 'fern' pattern (Pl. 8, fig. 6), a characteristic feature of which is a green line separating the vein from the yellow banding. As this trend progresses, the yellow areas become larger and some transparent lesions may develop, while the green pales so that the foliage of trees in the chronic phase represents a general yellowish green appearance, often with the main veins lined with green.

In the young leaf soon after emergence from the bud, the veins are bordered by a narrow red band (Pl. 9, fig. 7). In the early stages of infection this forms a fine red network over the lamina; later this pigment is restricted to larger veins and may form a red fern pattern. When the leaf becomes mature and develops chlorophyll the red colour disappears entirely (cf. strain C). As red vein-banding occurs in both healthy and diseased cacao types with red pigment (genetical) in the leaf the use of unpigmented types like the West African Amelonado is obviously advantageous for early diagnosis.

Pod symptoms. Soon after infection, frequently before leaf symptoms are visible, green (unripe) pods develop a darker green mottle. In later stages of infection this is overlaid by dark red marbling or blotches, the surface becomes smoother, and the pod rounded, often to the extent of being spherical instead of the long ovoid of the Amelonado type (Pl. 9, fig. 8). When the pod ripens to yellow the green mottle persists. Such pods are smaller and contain only about half the weight of beans of a normal pod; the beans are flatter, often containing cotyledons paler than normal.

Virulence. In addition to these symptoms, strain A has a pronounced effect on the vigour of the cacao plant. This is sometimes noticeable even in the first symptom-bearing leaves, which may shrivel and fall before maturity. Early senescence and shedding of leaves become more marked in later flushes; leaves which are held become progressively smaller at each succeeding flush until only one-half or one-third normal length. As growth weakens, internodes are reduced until finally the

apical bud dies and die-back proceeds down the tree. A number of axillary buds are stimulated to abortive growth before the tree is killed, usually within 2 years of infection though this may be shortened by adverse soil conditions or insect attack.

Experimental characteristics

Cross-immunity reactions. The ability of other strains to protect a plant against infection by strain A is discussed in the appropriate section of the description of each strain.

Latent period. The interval between inoculation and the appearance of symptoms has varied greatly in experimental transmissions. The chief factors influencing the latent period are: (*a*) the interval between inoculation and subsequent growth flushes; (*b*) the size of the plant.

Leaf symptoms are dependent on new growth and if a flush develops within 2 or 3 weeks of infection it is usually symptomless. There may be an interval of 2 months before the next flush, giving a latent period of about 3 months. With fifty seedlings about 3 ft. tall, the mean latent period from graft infection has been 77 days (S.E. = 4·7); with mature trees it has been about 150 days; with seedlings less than 2 ft. tall 30 days is not uncommon. This difference between seedlings and trees is partly due to the difficulty of seeing the preliminary symptom in the leaf-canopy 12 ft. above the ground.

In attempts to shorten the latent period, experiments were carried out on the site of grafting and on the number of infecting buds. No difference in latent period was found between 1-year old seedlings infected with one, two, three or four buds; nor was the latent period affected in similar seedlings when infecting grafts were placed on the stem at ground level or as high as possible.

Translocation of virus. An experiment similar to that described by Kunkel (1938) for peach viruses showed that the virus moves from the infecting graft to the stock in less than 24 days after grafting. Seedlings were decapitated immediately below the graft at intervals of 2 days; no virus was transmitted to the seedlings decapitated up to 22 days after grafting, but 24 days was sufficient for transmission to occur.

This technique ensures that all scion tissue is removed, but should the virus move upwards on entering the stock it would be removed by decapitation. The alternative method of removing only the graft-piece gave 10 days as the minimum time for virus to pass from scion to stock.

The effect of ring-barking on the translocation of the virus was investigated by the use of fifteen double-stemmed mature trees. With ten trees, one stem was completely ring-barked and an infected bark-graft inserted above the ring-bark on five, below it on five. The other five trees were not ring-barked and were infected by a bark-graft on one stem; in these symptoms appeared on both stems. No symptoms appeared above the ring-bark on the trees with grafts inserted below it, nor did the virus pass downwards in two trees with grafts above the ring-bark. In three trees, however, the virus did pass downwards past the ring-bark (unless carried across by

vectors). The absence of virus from the symptomless part of each tree was confirmed by grafts to seedlings 14 months after the trees were infected.

Root transmission. The ability of the virus to invade the root was investigated. Using the same technique as for twinning (Posnette, 1942, 1943*b*), cacao seedlings were bisected so as to be joined only at the tip of the tap-root. When sufficiently grown, one-half of each of sixteen 'paired' seedlings was infected by a bark-graft. Symptoms developed on both grafted and ungrafted halves of fifteen plants, but only on the grafted half of one; when this plant was lifted, it was found that the root-junction had been broken, and although the roots of the twins had been in close contact there was no union.

The virus has also been transmitted by grafts from roots of infected trees to stems of seedlings.

Seed transmission. The first seed-transmission trials revealed a striking difference between seedlings grown from healthy trees and those from infected trees, many of the latter being stunted and chlorotic, with shortened internodes and axillary bud-stimulation. This was recorded as evidence of seed transmission of the virus (Posnette, 1941). The problem has now been investigated further by S. H. Crowdy and the writer, and it has been shown that seed transmission does not occur. Of 5577 beans planted from trees infected with strain A, 80% germinated (compared with 96% of controls) and no seedling developed mosaic or swellings. Stunted seedlings did not transmit virus when grafted to healthy seedlings, but the former developed virus symptoms when graft infected.

There is little doubt that the dwarfing effect is the result of the disturbed metabolism of the parent tree and this was investigated by comparing the growth of seedlings from 300 beans from infected trees with 300 of comparable weight from healthy trees. It was found that stunting was not a reflexion of bean weight. Seedlings from healthy trees were generally larger than those from infected trees; the mean difference in height was 1·0 cm. (sig. $P=0{\cdot}04$) and in girth 1·3 mm. (sig. $P=0{\cdot}01$) at 20 weeks after planting when the average height of all seedlings was 33 cm. Fifty-four seedlings from infected trees were classified as 'dwarfs', being less than three-quarters the height of the control seedlings growing in the same pot.

The effect of parental infection was also reflected in the colour of the cotyledons. Using Amelonado seed in which cotyledons are usually dark purple, 234 bore pale cotyledons out of 271 from infected trees, compared with 42 seedlings out of 269 from healthy trees.

Staining reaction. Infection with strain A increases the speed at which sections of woody stem stain in acidified methyl alcohol. This test is similar to that used for phoney peach (Hutchins, 1933).

Heat inactivation. The virus was not inactivated in budwood immersed in water at 45° C. for 30 min., at 50° C. for 12 min., or at 52° C. for 10 min.

Rate of spread. The two types of dispersal, radial and sporadic, have been described (Posnette, 1943*c*). The areas of observed outbreaks in African-owned plantations

have enlarged approximately three times in 3 years, eleven times in 5 years and eighty-eight times in 7 years. On the 70 acres of cacao which have been under observation at Tafo since 1937, the number of new outbreaks found annually rose from ten in 1939 to forty in 1942, since when between forty and forty-eight have occurred each year in spite of prompt rogueing which has controlled the radial spread of each outbreak. The surrounding cacao farms, being heavily infected, were presumably the source of virus for these sporadic outbreaks.

Attenuated strain A

The existence of a mild form of strain A was first discovered from work on apparently healthy trees found surviving on cacao farms devastated by swollen shoot. Clones developed from such trees did not suffer when attempts were made to infect them with strain A by grafting, and it was thought at first that such trees might be genetically resistant to swollen shoot. However, when it was found that bark-grafts from such clones to susceptible seedlings conferred on these a similar resistance to subsequent infection with strain A, it was apparent that the survival of the trees was not because they were genetically resistant to infection, but because they were protected against the virulent strain by the presence of a less virulent one. This attenuated strain now seems to be becoming established in new plantings. It has been found in many seedlings, which grow strongly in spite of infection, on farms replanted after devastation (Posnette, 1945*a*).

Symptoms

Swellings. Swellings are rarely induced and when present are usually internodal on fan branches and so small as to give the appearance of slight constrictions at the nodes.

Leaf symptoms. Leaf symptoms are usually inconspicuous and confined to one or two leaves on the plant. In the first flush of leaves after infection, the yellow veinlet-banding and flecking resembles the preliminary or 'rapid-transmission' symptom of strain A (Pl. 8, fig. 2), but subsequent flushes seldom develop more than a faint 'fern' pattern and are usually symptomless. The fine-grain mosaic and transparent lesions of virulent strain A are seldom formed and when it occurs the mosaic is composed of tiny pin-point yellow dots. Red vein-banding is faint and often absent even from symptom-bearing leaves.

Pod symptoms. None has been recorded.

Virulence. Leaf-shedding and dieback are not caused by this virus and except for the shock effect of initial infection there seems to be little, if any, reduction of leaf-size or growth rate. After 2 years' infection, seedlings infected with attenuated strain A are as large and as vigorous as the uninfected controls. Any effect on yield has yet to be determined, but trees known to have been infected for 4 years are still producing average yields.

Experimental characteristics

Cross-immunity reactions. The ability of this strain to protect a plant against invasion by virulent strain A has already been reported (Posnette, 1945*b*). As this is important in selecting for resistance, some seemingly resistant selections being protected by previous infection with a mild strain instead of being inherently resistant, the detailed results of cross-immunity and other experiments with attenuated strain A will be described in a later paper.

Latent period. As for virulent strain A.

Description of strain B

Common name	Swollen-shoot strain B.
Scientific name	*Theobroma* virus 1 B.
Synonyms	Bisa strain; mild strain.
Type locality	Bisa, Krobo district, Gold Coast (1938).
Vectors	*Pseudococcus njalensis* Laing (Box, 1945); *Ferrisia virgata* Ckll. (Posnette & Strickland, A. H., unpublished).

Symptoms

Swellings. Swellings are formed on branches and roots as with strain A, but are more pronounced and usually more elongated in proportion to their diameter. The growth of the apical bud is sometimes checked, but usually continues and further growth may mask or even obliterate the swelling.

Leaf symptoms. Leaf chlorosis has never been observed in natural outbreaks, but it not infrequently occurs in experimental transmissions. It is confined to a single flush of leaves, usually the second or third after inoculation, and resembles the preliminary symptom of strain A in that the network of fine veins is cleared and banded with yellow (Pl. 8, fig. 2). There is a tendency for the clearing to be less complete and the yellow more diffuse, giving the impression of general slight chlorosis over the entire lamina. Subsequent flushes are completely symptomless and not accompanied by any diminution in leaf size or defoliation. Faint red vein-banding in young leaves may occur but is usually absent.

Pod symptoms. None has been recorded.

Virulence. This virus has not caused any appreciable reduction in growth or yield after as much as 4 years' infection.

Experimental characteristics

Cross-immunity reactions. The ability of this strain sometimes to protect a plant against invasion by strain A has been reported (Posnette, 1943*b*).

Latent period. The interval between infection and the appearance of leaf symptoms is influenced by the same factors as for strain A. With thirty-two seedlings the mean was 113 days (S.E. = 8·7). The leaf symptoms on mature trees are not clear enough for accurate observation, so that diagnosis depends on swellings, and the time at

which a swelling becomes recognizable depends on the experience of the observer. The average latent period before the production of obvious swellings has been about 220 days on mature trees.

Seed transmission. 2813 beans from B-infected trees have been planted. Germination was 89%, compared with 96% for controls; no seedling has developed swellings and none was stunted (cf. strain A).

Heat inactivation. This virus was not inactivated in budwood immersed in water at 45° C. for 30 min. or at 50° C. for 10 min.

Staining reaction. Sections of woody stems infected with strain B stain in acidified methyl alcohol more rapidly than comparable sections of healthy stems.

Rate of spread. There are no figures for the rate of spread of strain B in natural outbreaks, but in inoculated plots spread has been much slower than that of strain A.

Description of strain C

Common name	Swollen-shoot strain C (?).
Scientific name	*Theobroma* virus 1 C (?).
Synonyms	Kpeve strain; mottle-leaf of cacao.
Type locality	Kpeve, British Mandated Togoland (1940).
Vector	*Pseudococcus citri* Risso (Box, 1945).

Symptoms

Swellings. No swellings have been observed either in natural outbreaks or in experimental transmissions of strain C.

Leaf symptoms. There is much less variation in the symptoms of strain C than in those of A; the preliminary symptom of fine vein-clearing is similar, but the accompanying yellow vein-banding is usually more conspicuous. The leaves of subsequent flushes show not yellow vein-banding but a conspicuous yellow mottle concentrated between the secondary veins, and leaving broad, dark green bands along each side of the mid-rib and of the secondary veins (Pl. 9, fig. 9). Within the areas bounded by these green bands, the chlorosis seems to be unrelated to the finer veins, so that, unlike the symptoms of other strains, no reticulate mosaic is produced; hence the name 'mottle-leaf' (cf. mottle-leaf of cherries; Reeves, 1941). The chlorotic areas vary in degree of transparency, tending to complete clearing in the centre and to opaque yellow at the edge.

Necrosis of the clear lesions, resulting in ragged-edge or 'shot-holing' of the leaf, is prevalent on certain clones but seldom occurs on others. Downward rolling of the leaf-edges and interveinal crinkling is more severe than with strain A.

There is no clear distinction into acute and chronic symptoms. Trees known to have been infected for at least 6 years still show the same intense mottle on each flush; no 'fern' pattern is produced.

The red vein-banding in the young leaf differs from that of strain A in that the red bands are much broader and often restricted to the secondary veins (Pl. 9,

fig. 10). Sometimes the red pigment is not restricted to vein-bands but occurs as a wash or mottle over most of the lamina. Unlike strain A symptoms, the red pigment persists for some time after the leaf is mature, intensifying the green so that a two-tone green mottle is produced.

Pod symptoms. A faint red blotching has been observed on the pods of some infected trees, but is not general. Although no deformation occurs, pod size may be reduced considerably.

Virulence. Strain C has a pronounced debilitating effect on most trees; the leaf-canopy becomes thinner and yield is reduced. Small branches may die, but trees are able to survive for many years, some without showing any obvious reduction in vigour.

Experimental characteristics

Cross-immunity reactions. The failure of strain C to antagonize strain A has been reported (Posnette, 1943*b*).

Latent period. The interval between graft-inoculation of thirty-two seedlings and the first symptom has averaged 77 days (S.E. = 3·7); with mature trees the mean was 190 days, significantly longer than with strain A.

Translocation of virus. The speed of transmission from scion to stock for strain C was compared with that of A by the seedling decapitation method. No transmission occurred when grafts were removed up to 20 days after budding, but 22 days was sufficient to transmit strain C.

In the ring-barking experiment of the type described under strain A, ring-barks usually succeeded in preventing the translocation of strain C. No virus passed downwards through the ring-bark on the five trees infected above it; the virus passed upwards through the ring-bark on one of the five trees infected below it (unless carried by vectors). The absence of virus from the symptomless parts 14 months after the ring-barked trees were infected was confirmed by grafts to seedlings.

Seed transmission. 5042 beans from C-infected trees have been planted. Germination was 79%, compared with 96% for controls; no seedling has developed leaf mottle and none was stunted (cf. strain A).

Staining reaction. The staining of woody stem sections in acidified methyl alcohol is either not affected or is delayed by strain C—the reverse of the strain A effect.

Rate of spread. The spread of strain C has been observed in the type outbreak on the Agricultural Station at Kpeve. When surveyed in November 1942, 312 trees were infected out of a total of 1318 in the plot; infection had increased to 514 trees by December 1944, and to 664 by July 1946. This represents the radial spread of the original outbreak, and in addition three 'satellite' outbreaks (totalling 136 infected trees) have developed in an adjacent plot and one infected tree was found in another plot about 100 yd. away.

Description of strain D

Common name	Swollen-shoot strain D.
Scientific name	*Theobroma* virus 1 D.
Synonym	Nkawkaw strain.
Type locality	Abuorso, near Nkawkaw, Kwahu district, Gold Coast (1942).
Vectors	*Pseudococcus citri*, Risso (Box, 1945); *P. njalensis* Laing (Posnette & Strickland, A. H., unpublished).

Symptoms

Swellings. Swellings are seldom formed, but occur occasionally as slight internodal inflations near the tips of fan branches.

Leaf symptoms. The preliminary symptom (clearing of fine veins) is rare with strain D. The first symptoms are usually pin-point yellow spots, evenly distributed over the leaf; as the leaf matures those spots are so masked as to become almost invisible. In later flushes, symptom-bearing leaves are few, and show a distinctive mosaic composed of both fine yellow dots and larger (2 mm.) cleared flecks (Pl. 9, fig. 11). This 'pepper and salt' mosaic which is evenly distributed over the lamina in the acute phase is gradually concentrated (with successive flushes) between the secondary veins leaving these and the mid-rib banded with green; it may recur during the chronic phase (in addition to a 'fern' pattern indistinguishable from that of strain A) with the transparent flecks adjacent to the mid-rib or secondary veins.

In the young leaf, the distribution of red pigment resembles that of strain C, forming either broad red bands along secondary veins or more often a mottle not related to the veins, but there is never narrow banding of the fine veins as with strain A.

Leaf necrosis rarely occurs with strain D, but in the acute phase cleared lesions sometimes become necrotic.

Pod symptoms. None has been recorded.

Virulence. Strain D appears to cause a slight reduction in leaf size, but no defoliation or dieback. The growth of seedlings is reduced; growth-flushes are short and stems thin. On mature trees this is reflected in a reduced canopy and unthrifty appearance which suggests a decline in yield; no yield data are available.

Experimental characteristics

Latent period. In graft-transmissions to seedlings, symptoms have usually developed on the second or third flush of leaves after infection. The shortest latent period was 40 days, but the mean for twenty seedlings was 128 days (S.E. = 10·1)—longer than for strains A and C.

Staining reaction. Strain D has not affected significantly the speed of staining with acidified methyl alcohol in tests, but insufficient tests have been made for a definite conclusion to be drawn.

Discussion

The descriptions given above indicate the difficulties of determining to which virus strain symptoms should be attributed after a single observation of an outbreak in the field. Leaf mosaic in the chronic stage of infection with strain A or D is similar, for instance, and the presence of young leaves in which the distribution of red pigment differs may be necessary to distinguish these strains. In large outbreaks, trees can be found in various stages of infection so that the complete range of symptoms can be studied. As it is not always possible to decide which are the acute and which the chronic symptoms, even this may not provide sufficient data for distinguishing strains which produce similar symptoms but at different stages of infection. Symptomless flushes interspersed between acute and chronic phases occur not only with strain D but are characteristic of certain other strains (to be described in a later paper) and form a valuable diagnostic character.

Many of the cacao viruses can be differentiated on symptoms with certainty only if the development of infection from acute to chronic phases is studied in experimental transmissions. The writer cannot support Mangenot's contention (Mangenot *et al.*, 1936) that the anatomy of leaf symptoms provides a valuable basis for the differentiation of viruses, since strains A, C and D each produce both transparent lesions of undifferentiated tissue and opaque lesions with completely differentiated tissues, and gradations in between. The proportion of each type of lesion produced by a particular virus varies between leaves of the same flush and especially between different flushes, so that it seems probable that differences between lesions reflect different local concentrations of virus in the developing bud. The pattern or arrangement of the lesions on the leaf, particularly their relationship to the veins, seems to be a more reliable diagnostic character than their anatomy.

I am indebted to Mr J. Nicol for taking the photographs. I am grateful to the staff of the Pathology Division of the West African Cacao Research Institute for their assistance, and particularly to Mr S. H. Crowdy who contributed the figures quoted under 'Seed transmission'.

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EXPLANATION OF PLATES

PLATE 8

Leaves photographed by transmitted light, times ¼ approximately

Fig. 1. Swellings on stems showing death of apical bud typical of virus 1 A infection.
Fig. 2. Preliminary symptom, vein-clearing and vein-banding.
Fig. 3. Enlargement of part of the same leaf as in fig. 2, showing clearing of microscopic veins.
Fig. 4. Fine-grain mosaic, typical symptom of acute stage of virus 1 A infection.
Fig. 5. Blocks of chlorotic areas between tertiary and fourth-order veins. Intermediate stage of virus 1 A infection.
Fig. 6. Fern-pattern typical of chronic stage of infection with viruses 1 A and 1 D.

PLATE 9

Fig. 7. Red pigment banding secondary and tertiary veins in young leaf typical of virus 1 A infection; transparent lesions are also present.
Fig. 8. Normal pod on left, infected pod on right, showing dwarfing, rounding and mottling typical of virus 1 A infection.
Fig. 9. Interveinal chlorosis with broad green bands along secondary veins, typical of virus 1 C infection.
Fig. 10. Broad bands of red pigment along secondary veins in proximal part, and 'wash' on distal part of young leaf, typical of virus 1 C and of 1 D infection.
Fig. 11. Acute symptom of virus 1 D, 'pepper and salt' mosaic with green bands along secondary veins.
Fig. 12. Healthy leaf.

(*Received* 25 *December* 1946)

Fig. 1

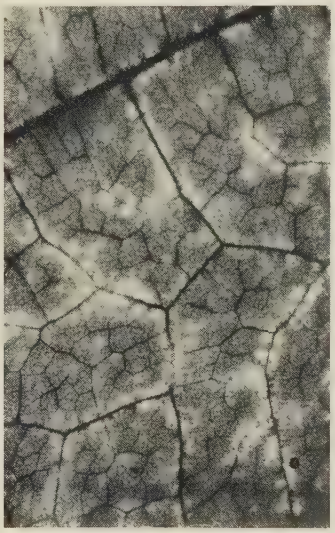

Fig. 3

Fig. 2

Fig. 4

Fig. 5

Fig. 6

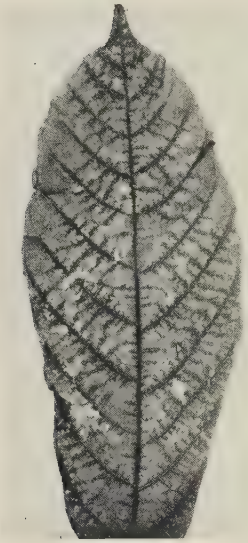

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

VIRUS DISEASES OF CACAO IN WEST AFRICA

II. CROSS-IMMUNITY EXPERIMENTS WITH VIRUSES 1 A, 1 B AND 1 C

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(With 2 Text-figures)

Experiments on cross-immunity reactions between three viruses attacking *Theobroma cacao* L. on the Gold Coast are described. A field trial involving 3 acres of graft-inoculated trees revealed some degree of protection afforded by *Theobroma* virus 1 B against infection with virus 1 A. The protection appeared to be more effective against insect inoculation than against graft transmission, being only temporary in the latter. Virus 1 C (probably unrelated but not to be called *Theobroma* virus 2 until more evidence is available) conferred no protection against virus 1 A.

The latent periods for these viruses were calculated from this experiment, which also provided data on their effects on yield. Virus 1 A reduced yield by 50% in the first year after inoculation and killed the trees in the second. Virus 1 B had no appreciable effect on yield; virus 1 C reduced yield by 50% in the third year after inoculation but there was no further decline in the fourth.

The rates of spread of these viruses were compared and significant differences demonstrated.

Introduction

The first paper in this series (Posnette, 1947) described the symptoms produced in *Theobroma cacao* L. on the Gold Coast by the cacao viruses 1 A, 1 B, 1 C and 1 D. That account constitutes an introduction to the present paper, which describes cross-immunity experiments carried out between 1940 and 1946 at Tafo, where the Central Cocoa Research Station of the Gold Coast Department of Agriculture was enlarged in May 1944, to become the West African Cacao Research Institute.

The ability of one virus to protect a host against systemic infection by another has been regarded as evidence that the two viruses are closely related, a relationship which is usually expressed by describing them as strains of a single virus. Conversely, the failure of one virus to antagonize another often means that the two viruses are unrelated and distinct; this converse does not always hold and seems to be influenced by the method of inoculation (Bawden & Sheffield, 1944). McKinney (1941) found with certain *Nicotiana* viruses that complete antagonism occurred only between virus mutants descended in direct line. Graft inoculations have been used successfully in cross-immunity experiments with peach viruses (Kunkel, 1936) and this method was adopted for cacao viruses. As extracted-sap inoculations have not been successful, and a technique for insect transmission had still to be worked out, grafts provided the only means of experimental inoculation.

The symptoms of viruses 1 A, 1 B and 1 C* are quite distinct (Posnette, 1947), but as the clearest differences lie in the presence or absence of certain symptoms, the interpretation of cross-immunity tests is easier when the viruses are inoculated in a certain order. For instance, if a plant is infected with virus B, it will develop swellings and no marked leaf symptoms. After subsequent infection with either A or C, leaf mosaic or mottle will disclose the presence of the latter viruses. If the plant is first infected with virus A and later with B, it would be difficult to tell whether swellings were caused by A or by B. Similarly, between viruses A and C, the clearest distinction lies in the presence of swellings due to A and their absence with C infection, which can be diagnosed on leaf mottle. The extreme virulence of virus A also makes it unsuitable for the primary inoculation.

Cross-immunity experiments

Between 1940 and 1942 three experiments were carried out on cross-immunity reactions between viruses A and B, and two similar experiments with A and C. In these experiments relatively small numbers (20–30) of plants were used, the majority being seedlings about 2 years old which were infected by grafting first with either virus B or C and later, at intervals up to 1 year, with virus A. These experiments showed clearly that virus C does not antagonize virus A, since symptoms of the latter virus developed on C-infected plants as rapidly as on the control plants inoculated with virus A alone.

The results with viruses A and B were variable; in one experiment B apparently protected against A, while in the others, virus A symptoms were merely delayed by prior infection of the plants with virus B. It was decided to lay out a large-scale experiment which would not only test the protective ability of the mild virus B against infection by the virulent virus A, but would also indicate the practical possibilities of utilizing this 'acquired immunity' effect, if substantiated, for minimizing the spread of virulent strains in mature cacao farms.

Yield and immunization experiment

This field trial was started in November 1942, with the following objects in view:

(*a*) To investigate the protective effect of virus B against infection by virus A: (i) when graft inoculated; (ii) when allowed to spread naturally by vectors.

(*b*) To find out the effect of the three viruses on the yield of cacao.

Layout. The trial was laid down on an area of comparatively uniform cacao, 15–20 years old, of the West African Amelonado variety in which there is little tree-to-tree variation in pod size. The layout consisted of three blocks, each of 1 acre and each divided into sixteen square plots. Treatments were allocated to each of these one-sixteenth-acre plots by randomization, there being two plots of each main treatment in each block, giving six replications. Owing to the irregular spacing, the number of trees per plot varied between 31 and 59 with a mean of 44·5; the experiment included a total of 2137 trees.

* Referred to below as A, B and C.

Each virus was inoculated by two bark-grafts on each tree; if the first grafts failed, a second pair was inserted 3 weeks later. The treatments were:

A	Virus A alone	CA	C followed by A
B	Virus B alone	BC	B followed by C
C	Virus C alone	CB	C followed by B
BA	B followed by A	O	Uninoculated control

The interval between the double inoculations in treatments BC and CB was 6 months. In treatments BA and CA two intervals (sub-treatments) were used, two plots (BA 1 and CA 1) in each block having 6 months, and the others (BA 2 and CA 2) 12 months between the B (or C) and the subsequent A inoculation. Inoculation of the plots carrying A alone was also carried out at two different times to coincide with the inoculation of A into the BA 1 and BA 2 treatments. The infection on the B and C plots therefore preceded that on the A 1 plots by 6 months and that on the A 2 plots by 1 year, so that all A 1 inoculations could be done simultaneously, and similarly all A 2 inoculations 6 months later (Table 2).

Records. The following records were taken for 4 years (1943–6):

(1) Date of appearance on each tree of (*a*) swellings (A or B symptom); (*b*) leaf mosaic (A symptom); (*c*) leaf mottle (C symptom). (2) Number of pods harvested per tree. (3) Number of pods harvested per plot.

For 3 years: (4) Weight of wet cacao from ten pods selected at random from each plot, all plots being recorded at the same main crop harvest.

Results

(1) *Symptom data*

Latent period

The detection of the first symptom is difficult on mature trees owing to the height of the canopy and the intermingling of the foliage of several trees. Often symptoms were noticed first on a sucker growing from the base of the tree, and as only some trees produced suckers, this increased the variation in apparent latent period. A further complication was the large scale of the experiment, which made it impossible to inspect each of the 2137 trees more than once a month. The figures obtained therefore do not represent the minimum latent period, but give a good indication of what can be expected in natural outbreaks under observation after rogueing has been carried out (Anon. 1945); and furthermore, as the same observer was employed throughout, comparisons between the latent periods of the viruses are legitimate.

There being little difference between plots in each treatment, two plots of each were chosen (in which the 'take' of the first grafts was most successful) for the calculation of the standard errors, and gave the following mean latent periods:

Virus A 154 days (S.E. = 3·6) to mosaic
Virus B 220 days (S.E. = 7·5) to swellings
Virus C 193 days (S.E. 4·4) to mottle.

Immunization effect

The experiment was designed to reveal any protection afforded by prior infection with either virus B or C against infection by virus A when:

(*a*) *Inoculated by grafts.* The symptom records do not reveal any gross immunizing effect by either B or C against A inoculated by grafts. In the BA plots, considerable tree-to-tree variation was apparent in defoliation and the time taken for symptoms of the A virus to appear, indicating a partial antagonism in some trees (which was reflected in the yield records, see Text-fig. 1) and little or none in others. After 2 years, however, there was no difference between the BA and the corresponding A plots. In the CA plots symptoms of the A virus appeared as rapidly and as generally as in the A plots, there being no indication of antagonism between A and C viruses.

(*b*) *Inoculated naturally.* From the symptom records of the natural spread of the A virus into adjacent plots there is strong evidence of antagonism between A and B viruses, and its absence between A and C. The figures for the invasion of A (up to 1946) into inoculated plots (Table 1) show that fewer trees in the B and BC plots became naturally infected with A than in the C and the uninoculated control plots. Treatment CB occupies an intermediate position, either because of the later time of the B inoculation or because of a weakening effect of C. The difference between the degree of invasion by A into the B and BC plots and that into the C and control is statistically significant at the 5% point ($F=5{\cdot}0$; $n_1=1$, $n_2=25$).

Since the proximity of an A plot would be expected to predispose a particular plot to invasion by the A virus, it should be mentioned that, in so far as the layout resulted in any differences between treatments in this respect, the balance favoured the invasion by A of the B rather than of the C and control plots.

Table 1. *Degree of invasion by virus A*

Virus inoculated 1942–3	Total no. of trees (6 plots)	No. of trees infected with virus A in 1946	% A infection	Difference
B	245	130	53·0	
BC	262	124	47·3	
CB	268	161	60·1	14·1*
C	273	209	76·4	
Control	258	173	67·1	

* Sig. $P=0{\cdot}05$.

(2) *Yield data*

During the course of the experiment the yield (in number of pods) of each tree was recorded individually. In the analysis of the yield data, those trees which became accidentally infected (through natural invasion by a virus not in that particular treatment) were omitted from the calculation of mean yield per tree, but included in the plot yields. Thus the decline of yield per tree can be attributed to the inoculated virus, while the decline in plot yield may be due also to invasion by other

viruses from adjacent plots; the trend of the plot yields therefore reflects any cross-immunizing effect.

Effect of viruses on yield

Text-fig. 1 shows the effect of each virus on yield expressed as pods per tree, most differences not significant at the 5% point being omitted for the sake of clarity. It will be noted that whereas virus B has not reduced yield, C has caused a 50% decline in 3 years; and that A reduced yield 25% in the first crop (1943 for A 1) after infection, 50% in the second (1944 for A 1, 1945 for A 2) and entirely stopped production in the third crop. The rapid decline in yield due to virus C was unexpected in view of the much slower effect of this virus on the apparent vigour of trees.

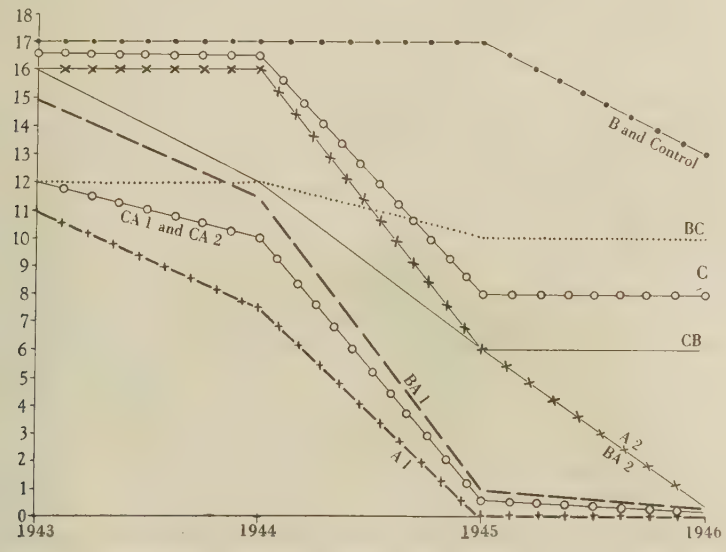

Fig. 1. Mean yield per tree (in pods).

Immunization effect

From the yields of the double inoculations (Text-fig. 1) it can be seen that the combination of viruses B and C reduces yield as rapidly as does C alone, and that C confers no protection against the A virus and may even intensify its effect. On the other hand, there are indications of partial protection by B in the difference between BA 1 and A 1 in the yield of the 1943 crop (6 months after infection) and also in that of the following year (sig. $P = 0{\cdot}02$). This protective effect does not appear in the BA 2 inoculations, and disappeared after 2 years from the BA 1 treatment.

The protecting ability of B appears to be more effective against virus A when spread by vectors than when graft-inoculated. This is supported by the decline in

the yields of plots caused by both inoculated and invading viruses shown in Text-fig. 2. In contrast to the maintained yield of uninfected *trees* (Text-fig. 1), the total yield of the control *plots* has been reduced by 66% because of the entry of virus A, while that of the B plots has declined 37%; this difference fails to reach statistical significance ($t = 2{\cdot}05$, $n = 10$) because one control plot, situated at a corner of the experiment and adjacent to virus B and C plots, escaped invasion by virus A. As was pointed out in the analogous section on symptoms, this apparent protection of B plots is not because they were isolated from A plots, and it is probably due to antagonism between the A and B viruses.

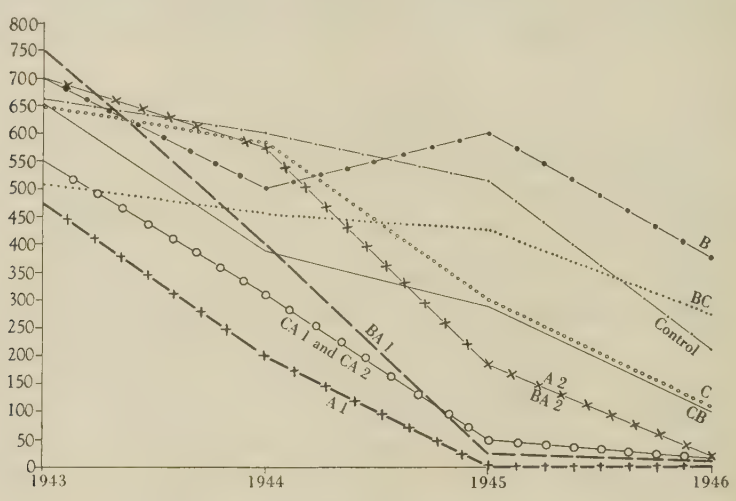

Fig. 2. Mean yield per plot (in pods).

Effect of viruses on bean weight

It was anticipated at the commencement of the experiment that although viruses B and C might have little effect on the number of pods produced per tree yet they might affect yield appreciably by reducing the weight of beans per pod. During the first year, the wet beans from ten pods per plot were weighed at three pickings well separated through the crop. The difference between the mean pod weights for each picking is significant; the extremes were 128 and 107 g. of wet beans per pod, representing a decline as the season advanced. In subsequent years the beans were weighed for all plots at the same picking, and so that the figures should be as comparable as possible from year to year, a picking near the middle of the main crop (November) was chosen.

The effect of the treatments on bean weight is given in Table 2. The effect of virus A in deforming the pod (dwarfing and rounding) is reflected in the significant reduction in weight of wet beans per pod. Virus C has slightly reduced bean weight, while B has had no appreciable effect.

TABLE 2. *Virus effect on bean weight*

Treatment	Date of inoculation	Weight of wet beans per pod (g.) 1943	1944	1945
Control	—	114	113	109
B	xi. 41	113	105	105
C	xi. 42	112	99	99
A1	iv. 43	105	77	*
A2	xii. 43	114	95	76
BC	xi. 42; v. 43	112	105	97
CB	xi. 42; v. 43	112	100	96
BA1	xi. 42; v. 43	105	83	*
BA2	xi. 42; xii. 43	107	103	76
CA1	xi. 42; v. 42	115	81	*
CA2	xi. 42; xii. 43	110	89	*

Critical differences ($P = 0{\cdot}05$) for main treatments = 3·5; for subtreatments = 5·0.

* Omitted owing to lack of pods.

Rate of spread

Owing to the different localities in which the three viruses have been found (Posnette, 1947) it has not been possible to obtain data on their rate of spread in natural outbreaks under comparable conditions. This experiment provided an opportunity for comparing the rates of spread of viruses A, B and C into the uninoculated control plots within the experimental area, and also into the surrounding border plots of healthy cacao. A control plot was subject to virus invasion from either two, three or four sides, according to whether it was situated at the corner, or the edge of the experiment, or was entirely surrounded by inoculated plots. The plots in the border were liable to invasion from one side only, and therefore have been treated separately.

Taking the six control plots together, the degree of invasion by viruses A, B and C up to 1946 was 67, 17 and 16% respectively, the spread of A being significantly greater than that of B and C ($t = 3{\cdot}05$, $n = 10$); this may be due partly to A masking the symptoms of B when double infections occurred. The double infection of a tree with two viruses was less likely to occur in the adjacent plots, only one side of each being in contact with an inoculated plot. Thirteen $\frac{1}{16}$-acre border plots were available with twenty or more trees and in similar proximity to plots infected with each of the three viruses. Out of a total of 534 trees, 219 became infected with virus A, ninety-six with B, and nineteen with C, the difference between A and B being significant at the 1% point ($t = 4{\cdot}57$, $n = 24$), and likewise between B and C ($t = 4{\cdot}43$, $n = 24$).

The rapid spread of virus A was expected from observations on natural outbreaks near Tafo, while virus C has spread comparatively slowly in the only known outbreak at Kpeve in British Mandated Togoland. That this difference is not entirely due to the virulence of virus A, which by causing rapid deterioration of the host might hasten the migration of the *Pseudococcus* vector, is shown by the spread of the apparently harmless virus B being greater than that of C. It would appear either

that C is less efficiently transmitted by its vectors, or that the latter were less active or less prevalent than the vectors of viruses A and B.

Discussion

A satisfactory feature of this 'immunization trial' is the general agreement between results obtained from symptom records and those from yield records. Both support the conclusion that virus B may afford a plant some protection against infection by virus A. The protection is variable from tree to tree and temporary against graft-inoculation of A; the evidence from double graft-inoculations is that virus B merely delays the multiplication of virus A in some plants. It seems probable that there are two factors to consider in interpreting this delaying effect—the distribution of the virus in the plant tissues and the concentration of the virus.

The protection acquired by a plant against one virus strain because of previous infection with a related strain depends on the continued presence of the first strain in the plant tissues, and it has been shown (e.g. Kunkel, 1934) that only those tissues containing the first resist infection by the second. The inconsistent results obtained with B–A double inoculations may be due to virus B not being systemic at the time of the A inoculation, so that virus A multiplies in tissues uninfected with B. The anatomical effect of the leaf symptoms of virus A (Posnette, 1947) provides evidence that it is not phloem-restricted, and suggests that it is more invasive and would quickly reach a higher concentration in the plant than virus B.

The concentration of some viruses in plant tissues at first increases rapidly after inoculation, reaches a maximum and then declines (e.g. Ross, 1941). The results of the 'yield and immunization trial' show a greater protective effect by B against virus A infection when the interval between the respective inoculations was 6 months than when it was 1 year, and this may indicate a fall in concentration of virus B after 6 months. This decrease in concentration is suggested also by the leaf symptoms of virus B, which occur on only one flush of leaves, subsequent flushes being symptomless (Posnette, 1947).

The protective effect of virus B appears to be greater against natural vector inoculation than against graft-transmission, which would be expected if the previous suppositions about the distribution and concentration of virus B in the plant are correct. A graft provides a constant source of virus, and also a greater area of contact for the virus to spread into uninfected tissue than would occur in an insect puncture, and furthermore, the latter would frequently be directed into actively growing tissue, in which the concentration of virus B might be expected to be greater than in the older tissue on which grafts are placed (Bennett, 1940).

It is clear that virus C does not protect against virus A whether graft or insect inoculated, and this raises the question of nomenclature. If cross-immunity reactions constitute a legitimate test for relationship, virus C should not be regarded as a strain of swollen-shoot (*Theobroma* virus 1) but as a distinct virus for which the name 'mottle-leaf of cacao' was suggested (Posnette, 1943). Although the symptoms

caused by virus C differ markedly from other cacao virus diseases, the writers are inclined to continue the use of the term 'virus 1 C', rather than adopt '*Theobroma* virus 2' for mottle-leaf, until the relationships of other cacao viruses have been investigated more fully and the classification of *Theobroma* viruses can be reviewed as a whole.

From the point of view of practical field applications, the most important finding recorded in this paper is the effect of the two 'mild' strains on yield. Virus A usually kills the tree within 2 years and a drastic effect on yield is inevitable; the failure of virus B to reduce yield appreciably was not unexpected, but it is clear from the decline in yield of the trees inoculated with virus C that the term 'mild' can be applied only to the visible effect on the tree's vegetative vigour, and even then is merely relative to the effect of virus A. A disease which causes a 50% reduction in yield of cacao warrants drastic control measures; the only mitigating factor being that the relatively slow rate of spread of virus 1 C suggests that it would not be difficult to control by rogueing.

This paper is published by kind permission of Mr O. J. Voelcker, Director of the West African Cacao Research Institute.

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STUDIES ON DANDELION YELLOW MOSAIC AND OTHER VIRUS DISEASES OF LETTUCE

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(With Plates 10 and 11)

The symptoms caused by dandelion yellow mosaic virus on cultivated lettuce, *Lactuca serriola* and *L. virosa*, are described and compared with those caused by lettuce mosaic virus.

Lettuce is much more susceptible than dandelion to the yellow mosaic virus; no infections of dandelion were obtained by mechanical inoculation and only three by aphides, whereas infection of lettuce is regularly obtained by aphides and by inoculation provided an abrasive is used. *Myzus ornatus*, *M. ascalonicus* and *Aulacorthum solani* transmitted dandelion yellow mosaic virus but not lettuce mosaic virus, whereas *Myzus persicae* transmitted the latter but not the former. *Nasonovia ribicola*, the common lettuce aphis, transmitted neither. Aphides became infective only after feeding periods of some hours on the diseased plants and ceased to be infective within an hour of the infective feeding. Their efficiency as vectors was not increased by a preliminary starving period, as happens with *Myzus persicae* and lettuce mosaic virus. Lettuce mosaic virus was found in most samples of commercial seed, which explains its prevalence; no evidence was found for the seed-transmission of dandelion mosaic virus and it is doubtful if it occurs, for infected lettuce are so severely affected that they rarely set seed.

Cucumber mosaic virus was isolated from naturally infected lettuce.

The occurrence of a previously undescribed virus affecting dandelion and lettuce has already been briefly reported (Kassanis, 1944). In the present paper the general properties of dandelion yellow mosaic virus are described and compared with those of lettuce mosaic virus, the most common virus found in lettuce.

Materials and methods

A number of lettuce varieties was used as test plants; Cheshunt Early Giant was used almost exclusively during the winter months, while during the rest of the year the other varieties tested were Trocadero, All the Year Round, Little Gem, Golden Ball, Sutton's Winter White, Tom Thumb, and Improved Unrivalled. No significant varietal differences were found in susceptibility and reaction to dandelion yellow mosaic virus.

Lettuces were inoculated when 8–12 cm. high; all the leaves including the very young ones were dusted with celite (Kalmus & Kassanis, 1945) or 400-mesh carborundum and then rubbed over their whole surface with inoculum.

Most of the aphis transmissions of dandelion yellow mosaic virus were made with *Myzus ornatus* (Laing), which successfully colonizes plants of *Lapsana communis* L., *Sonchus oleraceus* L., *Taraxacum officinale* Web. and *Plantago* sp. Other species of aphides used were *Myzus persicae* Sulz., which was raised on turnips, *Nasonovia*

ribicola Kalt. (*Myzus lactucae* Schz.), *Aulacorthum* (*Myzus*) *circumflexum* Buckton, *Aulacorthum solani* Kalt. (*Myzus pseudosolanii* Theo.), *Macrosiphum solanifolii* Ash. (*gei* Koch partim) which were raised on lettuces, *Myzus ascalonicus* Doncaster on onions, *Doralis* (*Aphis*) *fabae* Scop. on broad-beans and *Pemphigus bursarius* L. taken from galls produced on leaves of poplar trees.

Symptoms

Under experimental conditions the first symptoms on dandelions appear as a few isolated bright yellow spots, 5–6 weeks after infection. A fortnight later the spots increase in number and rings, oak-leaf patterns, and other designs are also formed which give the vivid yellow mottle characteristic of the disease (Pl. 10, figs. 1, 2). No stunting or deformation of leaves of infected dandelion plants has been observed and apart from the mottle their growth seems normal.

On lettuce the symptoms appear more quickly than in dandelion and are much more severe. Six to ten days after infection the young leaves show a bronze veinal necrosis. The laminae become covered uniformly with a fine network of veinal and interveinal necrotic etching, which partly consists of minute broken or entire rings. Later the necrosis disappears and the leaves become thick, blistered and stunted but usually without any mottle; the veins also become pronounced and shiny. Stunting is severe, accompanied by much leaf malformation, especially during autumn and winter. When plants are infected as seedlings little further growth occurs (Pl. 10, fig. 4).

A variation of the above picture was observed on three occasions when the veinal necrosis was succeeded by a veinal chlorosis which gradually developed into a very bright yellow mottle. Some of these plants later showed all the patterns which are common in infected dandelions (Pl. 10, fig. 3). This type of symptom was observed on two different varieties of lettuce.

On outdoor lettuces the disease is so destructive that plants are rendered valueless (Pl. 10, fig. 5). Necrosis is much more general and severe than under glass. Young lettuces are completely crippled, while on those which have already begun to heart severe necrosis usually starts in one outside leaf and spreads rapidly to the young inner leaves, which eventually rot as a result of secondary fungal infection. On individual leaves necrosis usually starts on one side of the lamina so that the midribs become twisted by differential growth (Pl. 11, fig. 1). If left in the ground, some plants may survive to bolt but the entire inflorescence becomes necrotic and little or no seed is produced. Symptoms at the bolting stage can easily be confused with those of lettuce mosaic which at this stage of the plant's life also produces severe necrosis on some varieties. On the younger plants, however, the two are readily distinguishable. As the symptoms of lettuce mosaic have been described in detail by Ainsworth & Ogilvie (1939) they need receive no further attention here.

In addition to the different varieties of *Lactuca sativa* L., two more species, *L. serriola* and *L. virosa*, were tested and found to be susceptible to both the viruses.

The first has dentate leaves and resembles the dandelion in appearance while the second has serrate leaves and looks somewhat like *Sonchus*. Twelve plants of each species were colonized with aphides from plants infected with each of the two viruses, using ten *Myzus ornatus* per plant for infection with dandelion yellow mosaic virus and five *M. persicae* for lettuce mosaic virus. All the plants of *Lactuca serriola* became infected with lettuce mosaic virus. The first symptom was a vein clearing on the younger and middle leaves, and this was followed by a green mottle (Pl. 11, fig. 3), similar in type to that observed on cultivated lettuce (Pl. 11, fig. 4). The edges of the laminae of the young leaves were curled downwards giving the infected leaves a much more slender appearance than that of healthy plants. Only three plants of *L. virosa* became infected and these gave a very violent initial reaction; the young leaves developed a severe veinal necrosis and the growing point was also affected (Pl. 11, fig. 2). Some of the middle leaves produced necrotic veinal reticulations, but the lower leaves showed only occasional necrotic spots. Later all three plants recovered somewhat but the new growth was pale, suspicious of mottle and showed some necrotic veinal spotting.

All ten plants of *L. serriola* became infected with dandelion yellow mosaic virus and developed a green mottle on the youngest leaves. A few of the young leaves also showed slight veinal necrosis. Eight of the *L. virosa* plants gave obvious symptoms. Most of the middle leaves developed bright yellow blotches, but a few showed shiny spots surrounded by etched rings.

Attempts were made to infect the following species with dandelion yellow mosaic virus, but without success: *Nicotiana tabacum*, *N. glutinosa*, *Calendula officinalis*, *Sonchus oleraceus*, *Cichorium intybus*, *Petroselinum sativum*, *Apium graveolens*, *Pastinaca sativa*. A number of plants have been seen in the field showing symptoms suggestive of virus infection and resembling those caused in dandelion and lettuce by yellow mosaic virus. All attempts to transmit a virus from such plants of *Lapsana communis*, *Sonchus oleraceus*, and common Chrysanthemum to lettuce, however, have failed.

MECHANICAL TRANSMISSION

Dandelion plants are extremely resistant to infection and no infection of this plant has been obtained by mechanical inoculation. Healthy dandelions have been rubbed with sap from infected dandelions and lettuces, with and without the use of both abrasives and reducing substance, but with no success. Lettuces, however, can be infected mechanically. The success of inoculation, however, varies from time to time, depending on the type of abrasive used, the size of plants at the time of inoculation and, above all, on the source of inoculum. Inoculations without an abrasive have given only an occasional infection and celite was more effective than 400 mesh carborundum in facilitating transmission. In one test nine out of twelve plants became infected with celite compared with two out of twelve with carborundum. The choice of leaf for obtaining inoculum is very important. Sap from infected

dandelions showing a pronounced yellow mottle is much better than sap from plants with less severe symptoms. Sap from infected lettuces gives much better results than sap from dandelions; the best inocula were obtained from necrotic lettuce leaves from plants infected 10–15 days previously. Even under the best conditions it seems that the virus content of sap is low; no infections have been obtained, even with the aid of celite, at dilutions greater than $\frac{1}{50}$ (Table 1), and in many tests the dilution end-point has been smaller than this.

TABLE 1. *Dilution end-point of dandelion yellow mosaic virus tested on two lettuce varieties with celite*

Dilutions ...	1/1	1/5	1/10	1/25	1/50	1/100
No. of plants infected	24	10	5	3	2	0
No. of plants used	38	20	17	20	20	27
% infection	63	50	29	15	10	0

Crushing the leaves in a solution of 0·5% sodium sulphite did not improve transmission; this reducing substance was found to facilitate the transmission of lettuce mosaic virus (Ainsworth & Ogilvie, 1939). Dandelion yellow mosaic virus seems to be sensitive to oxidation, however, for mixing the inoculum with an equal volume of 0·005 mol. $K_3Fe(CN)_6$ reduces infectivity considerably. On two occasions the passage of infected lettuce leaves through a domestic meat mincer yielded dark brown sap, which was not infective.

With such difficulty in obtaining mechanical transmission of the virus it has not seemed worth while to attempt to determine such properties as thermal inactivation point and longevity *in vitro*. The results of any such tests would depend so much on the type of leaf used to obtain inoculum and the susceptibility of the test plants that no significance could be attached to the results as indications of intrinsic properties of the virus.

APHIS TRANSMISSION

With aphides as with mechanical transmissions, dandelions are very resistant to infection. Only three transmissions out of 100 attempted to this plant were successful; these were with *Myzus ornatus* using fifteen aphides per plant. The virus, however, has been transferred by aphides from a naturally infected dandelion to lettuce and then returned to dandelion without any apparent change in the symptoms.

With lettuces, on the other hand, transmission by aphides is readily obtained, especially if the insects are fed on leaves showing the early necrotic etching. From a variety of species which was tried, *M. ornatus*, *Aulacorthum solani* and *Myzus ascalonicus* (Doncaster & Kassanis, 1946) can transmit dandelion yellow mosaic virus efficiently if fed for 1 day on the infective source. This is shown in Table 2, which also gives the results of a comparative transmission of dandelion yellow mosaic and lettuce mosaic virus. With the latter the aphides were first starved for

4 hr., then fed on the infective source for 3–5 min., such treatment gives 100% infection using five *M. persicae* per test plant. It can be seen that the vectors of dandelion yellow mosaic virus did not transmit lettuce mosaic, and *M. persicae*, a good vector of lettuce mosaic, has never transmitted dandelion yellow mosaic. It has been suggested (Ainsworth & Ogilvie, 1939) that *Nasonovia ribicola* Kalt might be a vector of lettuce mosaic. This aphis was tested as a vector on many occasions without any success, and field observations made in the vicinity of Rothamsted have shown that it can be present in very great numbers without any spread of mosaic.

TABLE 2. *Transmission of dandelion yellow mosaic and lettuce mosaic viruses by different species of aphides*

Species of aphis	No. of plants infected / No. of plants used: Dandelion yellow mosaic	Lettuce mosaic
Myzus ornatus (Laing)	30/46 (15)*	0/10 (15)
Nasonovia ribicola Kalt.	0/42 (20)	0/32 (20)
Myzus persicae Sulz.	0/32 (20)	15/15 (10)
Aulacorthum circumflexum Buckton	0/10 (20)	—
A. solani Kalt.	13/30 (20)	0/10 (25)
Myzus ascalonicus Doncaster	14/30 (20)	0/38 (25)
Macrosiphum solanifolii Ash.	0/10 (15)	—
Doralis fabae Scop.	0/10 (15)	—
Pemphigus bursarius L.	0/10 (15)	—

* Number of aphides transferred to each test plant.

The vectors of the two lettuce viruses both lose their infectivity in about 1 hr. after they have left the source of infection. In one experiment *Myzus ornatus* were fed for 1 day on an infected lettuce and then twenty individuals were placed on each of ten test lettuce plants. After 2 hr. feeding these aphides were removed to a second lot of ten test plants, where they remained for 24 hr. Seven out of ten were infected in the first lot and none in the second. In a similar experiment, after 12 days of infective feeding, the aphides lost their infectivity in the first hour of feeding on the healthy test plants.

In experiments with lettuce mosaic the results were similar. *M. persicae* were starved for 4 hr. and then fed on an infective leaf for about 5 min. Before transference to the test plants, they were fed on detached healthy leaves for periods of 0, 1 and 2 hr. Ten aphides were used on each test lettuce. Aphides from the first group infected all the test plants but no infections were obtained with aphides of the other two groups. In another experiment infective aphides were fed on succeeding test plants at intervals of 30 min. Only the first lot of plants became infected.

With both viruses, the vectors remain infective for a longer time if they are starved after their infective feeding than if they are allowed to feed continuously. Table 3 shows the longevity of both dandelion yellow mosaic virus and lettuce mosaic virus in starved aphides. *M. ornatus* were fed for 1 day on a lettuce leaf infected with

dandelion yellow mosaic virus. *M. persicae* were starved for 4 hr. before feeding for 5 min. on a leaf infected with lettuce mosaic. All aphides were then starved for different lengths of time before they were finally placed on the test plants. Each test plant was colonized with fifteen insects.

TABLE 3. *Longevity of dandelion yellow mosaic and lettuce mosaic viruses in their vectors when starved*

Starving time	0 hr.	1 hr.	2 hr.	4 hr.	8 hr.
Dandelion yellow mosaic virus	6/10*	6/10	4/10	4/10	2/10
Lettuce mosaic virus	15/15	6/7	13/15	8/8	8/8

* Numerator, number of plants infected; denominator number used.

In their inability to survive for long in their aphis vectors and in surviving longer in fasting than in feeding insects, both viruses resemble the type which Watson & Roberts (1939) have called 'non-persistent'. The viruses these authors place as 'non-persistent' have other characteristics in common; the vectors become infective after 2–5 min. feeding on a source of virus and their efficiency is greatly increased if they are starved for 3–4 hr. previously to the infection feeding. Lettuce mosaic virus clearly falls in this type, but dandelion yellow mosaic virus differs in that its vector *M. ornatus* does not respond to starving and its efficiency in transmitting the virus increases with the length of infective feeding time. This is shown in Table 4, where the results are presented as totals of five separate experiments made with fifteen *M. ornatus* per plant on four different varieties of lettuces.

TABLE 4. *Effect of preliminary starving and period of infective feeding on the efficiency of* Myzus ornatus *in transmitting dandelion yellow mosaic virus*

Starving period before infective feeding	4 hr.	None	None	None	None	None	None	None	None
Infective feeding time	2 min.	15 min.	1 hr.	2 hr.	4 hr.	7 hr.	1 day	2 days	3 days
No. of plants infected	0	0	1	2	11	4	23	5	2
No. of plants used	30	10	16	20	29	10	38	10	10
Percentage of infection	0	0	6	10	38	40	60	50	20

It can be seen that starving has no effect and that the maximum infectivity is obtained after the aphides have fed for 1 day on the infected plants; longer periods result in a reduction of efficiency. Recently Watson (1946) uses the response of the vectors to preliminary fasting as the main criterion in dividing viruses into 'persistent' and 'non-persistent' and not, as formerly, the length of time during which they remain infective; on this basis she classifies dandelion yellow mosaic virus as a member of the 'persistent' group. It is, however, doubtful if this is justified. It is more likely that the differences which exist between dandelion yellow mosaic virus on the one hand and lettuce mosaic virus and the other viruses of the 'non-persistent' group on the other, are quantitative rather than qualitative. From transmission tests there is evidence that dandelion yellow mosaic virus exists in a very low concentration in infected plants. Transmission by rubbing is fairly difficult and

under the best conditions of insect transmission fifteen aphides per plant will give an average of only 60% infection (Table 4), while lettuce mosaic virus can easily be transmitted by sap and five or even fewer aphides produce 100% infection. Other viruses of the 'non-persistent' group are even easier to transmit. It may be, therefore, that aphides fed on plants infected with dandelion yellow mosaic require a correspondingly longer time to acquire an infective dose of virus; consequently any beneficial effects on the aphides that might be produced by starving have disappeared during the period of feeding on the source of infection that is needed to make the aphides infective.

Seed transmission

In the course of these experiments lettuce mosaic has occurred quite regularly in plants raised from commercial seed. With the exception of seed of Cheshunt Early Giant, which has always given healthy plants, all the other varieties used in this work produced 2–4% infected seedlings. When lettuces of different varieties were raised from seed collected from infected plants an average of 4% infected seedlings was obtained suggesting that to obtain a level of as high as 2–4% infection in commercial seed, the seed growers' crops must be heavily contaminated.

Infected dandelions do not seem to be affected at all seriously by dandelion yellow mosaic virus and produce normal flowers and seeds. Over fifty dandelions raised from seed taken from an infected plant were healthy. Lettuces, however, suffer so seriously that it is difficult to secure seed from infected plants. Inflorescences are usually destroyed by necrosis or produce very few seed. Fifty plants raised from seed from an infected lettuce (var. Trocadero) were found to be healthy, but on such slight evidence seed transmission cannot be ruled out, though it can be of no practical importance.

Field transmission

Although dandelion plants are difficult to infect experimentally, infected plants are fairly common in the vicinity of Rothamsted and have also been obtained from several other districts. This frequency of occurrence may mean that there is a more efficient vector than those found in this work. On the other hand, a perennial plant has many opportunities of becoming infected and a virus that does such little harm as yellow mosaic could persist indefinitely in infected plants, and these facts probably account for its survival and relatively common occurrence.

In lettuce, dandelion yellow mosaic virus is much less common than lettuce mosaic, but it has been found in many parts of the country and on occasions affecting considerable number of plants. Moore (1946) has recorded what is known of its occurrence in Britain. Spread is much more rapid in lettuce than in dandelions but before an outbreak can occur virus must come into the plot. Most outbreaks have been in the vicinity of infected dandelions, but the possibility of additional perennial hosts also exists.

Small-scale field trials have been made to compare the rates of spread and the effect of the two viruses in lettuces. Spread of dandelion yellow mosaic virus was followed in a plot containing 150 lettuces of five varieties. When the plants were approximately 15 cm. high a row of ten infected lettuces, infested with the three aphides *Myzus ornatus*, *Aulacorthum solani* and *Myzus ascalonicus*, was planted across the middle of the plot. The first sign of spread was observed 13 days after planting the infectors, on a plant in an adjacent row. After 3 weeks eight plants in the rows adjacent to the infectors showed symptoms and after a month every plant in these two rows had become infected. After another 2 weeks more than 100 of the 150 plants in the plot were infected and showed acute necrotic symptoms. The few infected plants which bolted produced inflorescences with leaflets and flowers acutely necrotic, and set no seed.

Spread of lettuce mosaic virus was followed in another plot containing six different varieties which had been raised in an insect-proof glasshouse. Before transplanting all the seedlings which came up infected were removed except for four, which were used as infectors. The plot contained 650 lettuces and infectors were planted in two pairs at the south end. Observation taken at intervals showed that all the spread was around those four infectors. The lettuces at the north side of the plot although near an allotment which contained infected plants remained healthy throughout. It was observed that the disease did not spread widely until after the plants had hearted, so that their market value was not greatly affected. Symptoms became more pronounced later when the plants began to bolt, for necrosis of the inflorescences was severe. By the time the plants were in flower half the plants in the plot were infected. Thus in the course of 12 weeks four originally infected seedlings led to the infection of over 300 plants. The results of these tests showed that the spread of both viruses is mainly a local phenomenon and suggest that the control of the diseases should be reasonably easy. To avoid dandelion yellow mosaic, strict control of weeds in the vicinity of the plot is the main consideration. To avoid lettuce mosaic, the use of virus-free seed is the obvious measure, for infections coming from outside the plot will be too few and too late to cause severe losses. Alternatively, control of the seed-bed, by rogueing all infected seedlings as soon as they are seen and the use of aphicidal treatments, would probably prevent the large outbreaks that are now common. In growing lettuces for seed production, however, extra precautions are necessary to avoid the introduction of virus from outside the plots and its subsequent spread. The first could probably be achieved by growing lettuces for seed production in isolation from other lettuce-growing areas, the second by careful and frequent inspection and rogueing of infected plants.

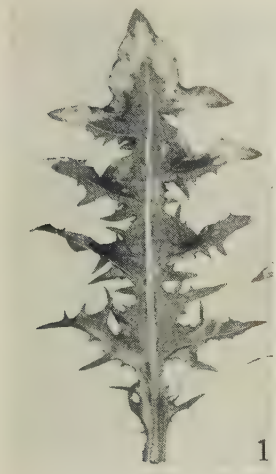
IMMUNOLOGICAL AND SEROLOGICAL TESTS WITH DANDELION YELLOW MOSAIC AND LETTUCE MOSAIC VIRUSES

No immunological relations have been found between dandelion yellow mosaic and lettuce mosaic. Lettuces infected with lettuce mosaic virus were reinfected by means of *M. ornatus* with dandelion mosaic. In a week the plants developed the typical bronze veinal necrosis of dandelion yellow mosaic virus over the already existing lettuce mosaic mottle. The two viruses combined crippled the plants to a greater extent than when affected with either virus alone. One month later *M. ornatus* and *M. persicae* were fed on the new growth of these plants with the double infection and then transferred separately to healthy lettuces. *M. ornatus* were given an infective feeding of 1 day while *M. persicae* were first starved and then fed on the infected plants for 2–5 min.; the first transmitted only dandelion yellow mosaic virus and the second only lettuce mosaic virus. It is obvious that the two lettuce viruses can successfully survive in the same plant.

Attempts were made to produce sera against the two viruses. Six injections of infective sap centrifuged at 8000 r.p.m. were made intraperitoneally into rabbits at weekly intervals. A week after the last injection, blood samples were drawn and the prepared sera were tested at dilutions of 1/5–1/160 against undiluted centrifuged sap from infected lettuces. No precipitin reaction was obtained. There is no evidence to indicate whether this is because both viruses occur in sap at too great dilution to give a visible precipitate with antiserum, or because they fail to elicit the production of antibodies either because of their instability or lack of antigenicity.

CUCUMBER MOSAIC IN LETTUCES

Hoggan (1933) has shown that lettuces are susceptible to cucumber-mosaic virus when inoculated, but apparently there is no record of any occurrence of natural infection. On three occasions during the course of the work diseased lettuces from the field sent to Rothamsted were found to be infected with cucumber mosaic. The strains were all of the types that are easily transmissible to both lettuce and tobacco by inoculation and by *M. persicae*. *M. ornatus*, an aphid not previously identified as a vector of cucumber mosaic, also transmitted it to lettuce quite readily. The efficiency of both vectors was increased by a preliminary fasting period followed by an infection feeding of only a few minutes. Under greenhouse conditions during the winter the first symptom in cos lettuce (var. Improved Unrivalled) was a chlorosis of the veins. Later this developed into a general chlorosis and infected plants became extremely stunted with small narrow leaves with the laminae curled downwards (Pl. 11, fig. 5). Older leaves showed some large necrotic spots which started and spread from the margins. Other varieties, however, produced a veinal mottle on the young leaves closely resembling the symptoms of lettuce mosaic. It is possible that cucumber mosaic occurs frequently in lettuce in the field, for it is widely distributed in many perennial hosts, but that it is usually diagnosed as lettuce mosaic virus. The latter is now undoubtedly the most common and important cause of virus disease of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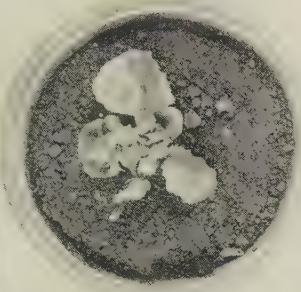

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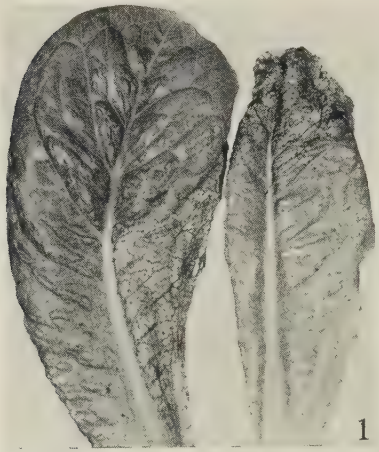
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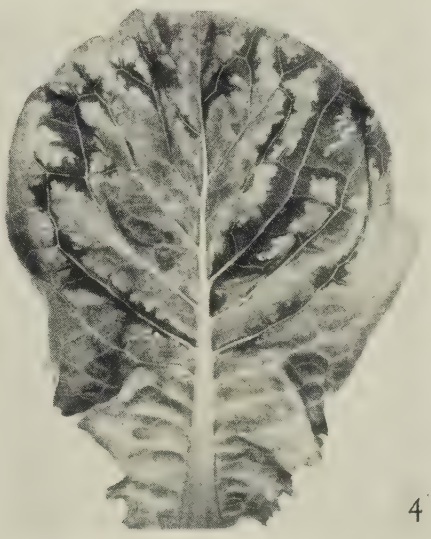
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lettuce. This fact is readily explicable by the regular transmission of lettuce mosaic virus through the seeds, which results in sources of infection within the crops at the start, whereas dandelion yellow mosaic and cucumber mosaic viruses probably have to come into the crop from outside sources.

I wish to thank Dr J. P. Doncaster for identifying the aphides used in this work, Mr L. Ogilvie for providing seeds of the two wild species of lettuce and Dr A. Kleczkowski for preparing the sera.

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EXPLANATION OF PLATES

Plate 10

Figs. 1, 2. Leaves from dandelions infected (1) artificially, (2) naturally with dandelion yellow mosaic virus, showing the yellow mosaic and patterns.

Fig. 3. Leaf from cabbage lettuce photographed 7 weeks after inoculation with dandelion yellow mosaic virus, showing bright yellow spots and patterns.

Fig. 4. The dwarfing effect of dandelion yellow mosaic on lettuce. Left-hand plant healthy, right-hand plant of same age infected 6 weeks earlier.

Fig. 5. Cos lettuce naturally infected with dandelion yellow mosaic virus showing a severe necrosis of the heart.

Plate 11

Fig. 1. Leaves from the heart of naturally infected cos lettuce showing a necrosis of the veins.

Fig. 2. *Lactuca virosa* infected with lettuce mosaic virus, showing severe necrosis of the young leaves and growing point.

Fig. 3. Leaf of *Lactuca serriola* infected with lettuce mosaic virus and showing a mosaic.

Fig. 4. Leaf from cabbage lettuce infected with lettuce mosaic virus and showing a dark green veinbanding.

Fig. 5. Effect of cucumber mosaic virus on lettuce (var. Improved Unrivalled) growing during the winter in the greenhouse. Left-hand plant healthy, right-hand plant infected 8 weeks earlier.

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EXPERIMENTS ON THE EFFECT OF CHLORINATED NITROBENZENES ON THE SPROUTING OF POTATO TUBERS

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(With Plate 12)

The addition of a small quantity of a dust containing pentachloronitrobenzene to clamps of certain early and second-early potato varieties has a marked effect in reducing the amount of sprouting which takes place over winter and in protecting the sprouts from damage by the fungus *Rhizoctonia Solani*.

A series of yield trials carried out over six seasons has shown that tubers which have been stored in clamps in presence of the dust give yields equal to those of chitted tubers.

A dust containing a tetrachloronitrobenzene applied to potatoes kept in a closed space represses sprouting to a very pronounced degree. The possible use of this dust for conserving ware potatoes is suggested.

Introduction

The work to be described here arose from that carried out by the writer over several years in connexion with the commercial cultivation of lettuce. It has been established that a proprietary preparation, Folosan, the active principle of which is pentachloronitrobenzene, can be used to reduce very materially the losses of lettuce plants caused by the fungi *Botrytis cinerea* and *Rhizoctonia Solani* (Smieton & Brown, 1940). Another strain of the latter fungus is commonly responsible for damping-off of Brassica seedlings, and it was soon learnt that a useful control of this trouble was also obtained with the same substance. An obvious experiment was therefore to test the effect of Folosan on sprouting potato tubers to see how far it affected the development of lesions on the young sprouts. These are caused by yet another strain of *R. Solani*, which commonly occurs in the form of small purplish black sclerotia (potato 'scurf') on the surface of the tubers and induces rust-coloured lesions on the sprouts, often leading to decapitation of the latter. The first experiment of this kind was carried out in the winter of 1940–1, with very distinct results, since when it has been repeated each year with greater or less elaboration. An account of these experiments, extending over 6 years, is given in this paper.

The experimental method consisted simply in setting up in the autumn two small seed-potato clamps of equal size, one as control and the other with a quantity of the dust blown among the tubers as the clamp was being prepared. An equal quantity of tubers was put out in trays to 'chit' in the usual way. The clamps were strawed, soiled up and left to over-winter. When opened in the spring, they typically showed a pronounced difference. The control clamp, if the variety used was of a kind which

readily sprouted, showed a mass of long thin sprouts on the outside, passing up through the straw and into and even through the soil covering. The whole clamp was more or less matted together through the growth of sprouts and roots, and almost invariably it was found that the sprouts among the mass of tubers were profusely marked with the characteristic lesions of *R. Solani*. The sprouts were typically long and spindly, or if short, they were so because they had been cut through by fungal action. The only clean sprouts were as a rule those which had grown up into the straw from potatoes of the outside layer, so that they did not come in contact with the surface of tubers on which sclerotia of the fungus occur. In marked contrast, the sprouts of the dust-treated clamp were much shorter, distinctly stouter and much more free of fungal lesions. Some quantitative data on this point will be given later.

In the control clamp, the vast majority of the sprouts were too long for planting, and if short, they were so because they were diseased. For planting purposes therefore they had to be removed. On the other hand, the majority of sprouts in the dust-treated clamp were short and stout and largely free of fungal lesions. With reasonable care in handling, tubers with such sprouts intact could be boxed and later planted. The general plan followed was therefore to make the three-fold comparison, in replicated yield trials:

(1) Tubers from control clamp, desprouted.
(2) Tubers from dusted clamp, with sprouts.
(3) Tubers from chitting trays.

Various additions were made to the general plan from time to time, as will appear later. The scale of the experiments, and the amount of replication in each experiment, varied from year to year according to circumstances; details are given in Table 3 below. The standard unit plot was a row, usually of 20–30 plants, according to the dimensions of the pieces of ground available.

Experimental results

Record of clamps

This was essentially the same for each year's experiment, so that one example will suffice. The particulars for the winter 1945–6 were as follows:

A. Var. Epicure. Scotch seed, set up on 30 Nov.:

(i) 16 stone in control clamp.
(ii) 16 stone in clamp with 3·5* oz. Folosan dust.
(iii) 16 stone in chitting trays.

The tubers in (ii) were lightly moistened with a watering-can as the clamp was being built in order to ensure that more of the dust, blown from a bellows, remained on the surface. This practice was always followed.

* This is the amount of dust delivered by the bellows. What proportion of it adhered to the tubers is not known. No attempt has been made to standardize dosage, and this in fact would not be practicable without special apparatus. As there is evidence that the active principle is volatile, the amount applied would, within wide limits, be a matter of little consequence.

B. Var. Great Scot. Once-grown seed, set up on 30 Nov.:

(i) 18·5 stone in control clamp.

(ii) 18·5 stone in clamp with 6·5 oz. Folosan dust.

(iii) 18·5 stone in chitting trays.

The two sets of clamps were opened on 3 and 16 April respectively. They showed the features, external and internal, which have been described in the preceding section. Pl. 12, figs. 1, 2, which relates to a similar experiment with var. Ninetyfold in 1942–3, illustrates the appearance of the clamps when stripped, and Pl. 12, fig. 3 shows sample tubers taken from the interior of the control and dusted clamps respectively.

The potatoes of each clamp were sorted into two lots according as the sprouts were (*a*) longer than 2 in. or short because the terminal bud had been killed by *R. Solani*, (*b*) shorter than 2 in. and free, or nearly so, from fungal lesions. The resulting data are set out in Table 1.

Table 1

Variety	Treatment	Weight (lb.) of tubers with sprouts: Exceeding 2 in. or badly diseased	Weight (lb.) of tubers with sprouts: Less than 2 in.
Epicure	Control	175·5	12·5
	Folosan	6	195
Great Scot	Control	196	36
	Folosan	28	226

Sprouts of the kind first mentioned were taken off, those of the second left on. The weights of sprouts removed from potatoes of the control and Folosan clamps of Epicure were 13 lb. and 5 oz. respectively, which further emphasizes the effect of the dust treatment.

The varieties which have been used are Ninetyfold, Epicure, Great Scot and King Edward. In each year usually two varieties were tested, a first early and a later variety. On account of the difficulty of obtaining Scotch seed, at least in good time, during the war years, and partly because such seed was sometimes badly bruised and infected with dry-rot, nearly half of the experiments were carried out with seed once-grown at Slough.

As the land available for planting was limited and the stock of Folosan dust could not be replenished during the war years, the clamps were small, varying from about $1\frac{1}{2}$ to 6 cwt.

The amount of sprouting shown when the control clamps are opened in the spring depends, of course, on the mildness of the winter, the variety, and the date at which the clamp is opened. In the one year in which King Edward was tested, sprouting was still backward in the control clamp when it was opened at planting time. The experiment with this variety was therefore carried no further. Presumably the same result would be obtained with such a variety as Majestic. With the three other varieties used, a condition similar to that shown in Table 1 was found in

each year—i.e. in the control pit the great majority of sprouts were too long or too diseased for planting purposes, whereas in the dusted clamps they were short and remarkably free of fungal lesions. Hence the standard scheme of planting-out as given above.

Yield trials

One illustrative experiment will first be given.

Var. Ninetyfold, clamped 7 Dec. 1942. Clamps, when opened on 24 March, showed the differences already described. As the seed was very unequal in size, a large and a small grade was selected and an eight-fold replication of each, in rows of thirty-five plants, was planted out on 25 March. In the forty-eight rows there were only ten misses.

In the earlier stages of growth it was clear that the rows from chitted seed were somewhat more advanced, and that those from the Folosan-dusted seed came next. At the time of lifting (21–23 June), no differences were perceptible in the tops. The yields (in lb./row) are given in Table 2.

TABLE 2

Treatment	Average weight per row of 35 plants: Large seed	Small seed
Control clamp	36·8	29·5
Clamped with Folosan	43·2	33·9
Chitted	44·5	35·9

Statistical analysis Large seed Fol. > Cont. and Chit. > Cont. ($P > 0{\cdot}01$):
Small Fol. > Cont. ($P = 0{\cdot}02$); Chit. > Cont. ($P > 0{\cdot}01$).

The seed which had been dusted in the clamp did not quite equal the chitted seed in yield, but was not far behind. Treatment with Folosan gave approximately two-thirds of the advantage given by chitting. The relevant comparisons, made over the past 6 years, are collected in Table 3. For purposes of comparison the yields given by seed (desprouted) from the control clamps are taken as 100, so that the percentage improvements obtained by using Folosan-treated and chitted seed can be read from the table.

If one ignores three experiments in 1944 which were vitiated by a late frost, there are altogether thirty-one comparisons of Folosan-treated and control seed. In one experiment the yields were the same; in all the others the Folosan-treated seed outyielded the controls. In twenty of these the increases were significant at the 20 : 1 level or higher.

The increased yield given by chitted seed over the control seed runs fairly parallel to the above. Averaging the results of twenty-seven experiments in which control, Folosan-treated, and chitted seed were compared (but excluding the three experiments which were spoiled by late frost), one obtains the following data:

Yield of control seed	100
Yield of Folosan-treated seed	120·8
Yield of chitted seed	120·0

Therefore under the varied conditions that obtained in five seasons' experiments and with three varieties of potato (two earlies, one second-early) the overall effect was that seed which had been sprouted in the clamp in presence of Folosan dust gave the same yield as chitted seed.

TABLE 3. *Yield records*

(Yield of control plants = 100)

Year	Variety	Planted	Lifted	Replications	Folosan-treated seed	Significance of increase	Chitted seed
1941	Great Scot	16 Apr.	27 Aug.	23	114·5	> 100 : 1	—
1942	,,	3 May	4 Oct.	11	112·4	> 100 : 1	111·9
	,,	5 May	7 Nov.	6	123·3	20 : 1–50 : 1	108·6
	,,	5 May	7 Nov.	5	110·7	5 : 1	107·2
1943	Ninetyfold	25 Mar.	21–3 June	8	117·4	> 100 : 1	120·9
	,,	25 Mar.	21–3 June	8	114·8	50 : 1	121·7
	Great Scot	1 Apr.	24 June	4	194·0	> 100 : 1	—
	,,		22 July	4	146·7	> 100 : 1	—
	,,		9 Sept.	5	146·8	> 100 : 1	—
1944	Ninetyfold	28 Mar.	14 July	15*	102·2	—	96·9
	,,	29 Mar.	19 July	4*	104·7	—	113·6
	Great Scot	30 Mar.	17 July	10*	102·2	—	115·1
	,,	26 Apr.	18 July	3	147·1	10 : 1–20 : 1	165·5
	,,		18 Aug.	3	141·6	20 : 1–50 : 1	161·3
	,,		18 Oct.	4	100	—	110·0
1945	Great Scot	5 Apr.	5 July	3	124·5	> 100 : 1	124·5
	,,		7 Aug.	3	123·3	> 100 : 1	128·8
	,,	6 Apr.	6 July	5	110·2	10 : 1–20 : 1	107·7
	,,	9 Apr.	17 July	3	106·4	2·5 : 1	111·6
	,,	9 Apr.	18 July	4	114·3	20 : 1	116·1
	,,	9 Apr.	18 July	2	112·7	20 : 1	109·4
	,,	11 Apr.	18 July	3	108·2	5 : 1	110·3
	,,	11 Apr.	19 July	4	104·0	2·5 : 1	104·6
	,,	11 Apr.	19 July	4	114·0	10 : 1	110·6
1946	Epicure	4 Apr.	1 July	4	145·2	> 100 : 1	84·8†
	,,		15 July	6	119·5	> 100 : 1	115·0
	,,		30 July	4	115·7	20 : 1	118·2
	,,	16 Apr.	11 July	6	143·8	> 100 : 1	138·4
	,,		25 July	8	123·8	> 100 : 1	122·9
	,,		9 Aug.	6	125·7	> 100 : 1	120·7
	Great Scot	18 Apr.	18 July	8	141·1	> 100 : 1	150·7
	,,		26 Aug.	8	117·8	10 : 1–20 : 1	121·3
	,,	30 Apr.	1 Aug.	4	121·9	10 : 1	117·3
	,,		5 Sept.	5	122·1	5 : 1–10 : 1	122·8

* Cut down by severe frost on 6 May.

† An aberrant result, cause unknown; P, 0·1–02.

TABLE 4

Date of		Yield (tons/acre)	
Planting	Lifting	Control seed	Folosan-treated seed
1 April	24 June	4·4	8·5
1 April	22 July	6·8	10·0
1 April	9 Sept.	8·2	12·1

As would be expected, the percentage increase tends to be greater the earlier the date of lifting. The most striking result was shown in the experiments with Great Scot in 1943. Table 4 gives the calculated yield in tons/acre for this experiment.

At the time of the final lifting the tops had completely died down so that the increase in yield obtained by sprouting the seed was a permanent one. In that year there was a midsummer drought which no doubt helped to confirm the preliminary advantage arising from the use of sprouted tubers. Nevertheless, it will be noted that even in 1946, in which there was no drought at any time, substantial increases were obtained even in comparatively late liftings.

The beneficial effect of the Folosan treatment arises simply from the fact that excessive sprouting is inhibited, with the result that the tubers do not have to be desprouted before planting. Subsidiary experiments have shown that tubers from the Folosan-treated clamp, after removal of the main sprouts, give the same yield as similarly treated control tubers, and conversely that when tubers from the control clamp, which carry short healthy sprouts, are planted they give the same yield as chitted or Folosan-treated tubers.

Throughout these experiments data of emergence have been recorded. Rows from chitted and Folosan-treated seed have invariably emerged some 10–14 days ahead of the control rows and this advantage has been readily visible in the height of the tops for fully a month afterwards. As between Folosan-treated and chitted seed, the advantage has sometimes lain with the one, sometimes with the other, and in no case has it been pronounced.

In the experiments of 1946, with vars. Epicure and Great Scot, the new form of Folosan known as Folosan DB905, the active principle of which is a tetrachloronitrobenzene, was also used. The rate of application was approximately the same as for the original Folosan, but the new preparation contains only 5% of active principle as against 20% in the other. Nevertheless, the effect of DB905 is a distinctly greater repression of sprouting. Whereas in the Folosan clamps the great majority of tubers had sprouts running up to 2 in. in length, and a proportion exceeding this figure, nearly all tubers in the DB905 clamps were unsprouted. The sprouted tubers were found almost exclusively at the bottom where they were in contact with damp soil.

Plantings from the DB905 clamps in the replicated series of 1946 gave slightly higher yields than the desprouted controls. For purposes of crop production therefore DB905 represses sprouting too much.

A small-scale replicated storage experiment, with tubers in closed tins, was carried out in the winter of 1945–6 according to the following scheme:

(1) Control.
(2) DB905 dusted on the surface of the tubers.
(3) DB905 placed in bottom of tin; tubers on gauze above this.
(4) Folosan as in (2).
(5) Folosan as in (3).

In this experiment equal amounts of the active principles were used. One series (three replications) was held in a warm greenhouse, the other in a cool shed.

The result was, as before, a much more pronounced repression of sprouting by DB905. With both preparations the effect produced was not obviously different according as the dust was or was not in contact with the tubers. Obviously, the effect arises from the volatile nature of the active principles. In the series kept under cool conditions, the tubers in the tins containing DB905 showed hardly any sprouting by the end of May at which time the experiment was concluded.

Discussion

The above experiments have shown that substantially the same yields are obtained with tubers taken from a clamp to which Folosan dust has been added as with chitted tubers. It remains to consider certain advantages and disadvantages of the dusting method.

So far the Folosan method has not been attempted with clamps of commercial size. There seems to be no *a priori* reason why similar results should not be obtained with large clamps and inasmuch as marginal effects would be proportionally less with the latter, one would expect better results. As the dust acts in virtue of its volatility, application as the clamp is being built would be simple.

The main difficulty would seem to be in the kind of sprouting which takes place in presence of the dust. Whereas in a chitting tray several eyes of each tuber produce sprouts, the dust-treated tubers usually sprout at one or two eyes only. Chitted tubers therefore can afford to lose some sprouts in handling; the dust-treated ones are more vulnerable. However, the experience of this work has shown that, with ordinary care, the sprouted tubers can be taken in handfuls from the clamp, placed several layers deep in trays and planted in the manner usual for chitted tubers, without serious loss of sprouts. Whether loss of sprouts would prove to be a serious disadvantage under normal commercial conditions can be found only by experience.

The obvious advantage is that, once the potatoes have been clamped in late autumn or early winter, no attention is required until planting time. In a chitting house, apart from the capital expense of a well-built structure there ar the precautions to be taken against spells of frosty weather, and the need to ensure sufficient illumination for all the chitting trays, a need which is met in practice by rearranging the chitting trays from time to time. Furthermore, the risk of aphis infestation which readily spreads virus diseases in the chitting house is minimized by the clamp method.

Although experiments with the new type of Folosan, which strongly represses sprouting, have been limited to 1 year, the results are so definite that they raise the question whether the new dust could not be used to hold back sprouting in clamps of ware potatoes. This would avoid much labour in desprouting during dressing as well as the shrivelling and loss of weight which are consequent upon sprouting. It would not be difficult to devise a method of application in which the dust as such

Fig. 1

Fig. 2

Fig. 3

did not come into contact with the surface of the tubers. Whether there would be any tainting of the potatoes, or any toxic effect of the chemical, are matters which further experiments must decide.

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EXPLANATION OF PLATE 12

Fig. 1. Appearance of control clamp (var. Ninetyfold) when opened in Spring.
Fig. 2. Appearance of dusted clamp (var. Ninetyfold) when opened in Spring.
Fig. 3. Top row, tubers from control clamp; bottom row, tubers from dusted clamp.

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EXPERIMENTS ON THE EFFECT OF CENTIMETRE-LENGTH ELECTROMAGNETIC WAVES ON WOOD-DESTROYING FUNGI

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In an attempt to devise a practical method of killing wood-destroying fungi, mycelia of *Coniophora puteana* and *Merulius lacrymans* were exposed to the beamed radiation from a standard Service radar transmitter transmitting pulses of radiation of wave-length 9·1 cm. Growth of *Coniophora puteana* on malt agar films on glass slides was checked. Many superficial hyphae were killed. Exposures up to 1 hr. did not kill the mycelium of either fungus when growing on malt agar slopes or plates, or wood blocks. The only obvious effect was a check in growth whenever drying of the mycelium occurred.

It is concluded that the rays have no lethal effect and injure the fungi only through drying of the mycelium due to the increase in temperature which they bring about.

Introduction

Ark & Parry (1940) have reviewed work on the effect on plants of high-frequency electric fields, chiefly in its application to agriculture. The papers quoted are mainly by Russian authors and are available to the writer only in review or abstract form. From these notices it appears that full information is seldom given on the exact nature of the electrical apparatus used. Any of the following points may be omitted from the description given: method of exposure of the experimental plants, wave-length of the radiation, field strength, temperature.

With regard to the fungi, where the method of exposure of the plant is described, it has been to place it between the plates of a condenser in an oscillator circuit. Wave-length, field strength and length of exposure have all been claimed as important in producing effects.

Tverskoy (1937) claimed selective killing of fungi (*Fusarium* spp.) in grain samples when exposed for 2 min. in a high-frequency electric field produced by an oscillating current (wave-length 5·6 m.).

Johnson (1932) obtained no effects when he exposed cultures of *Fusarium batatis*, *Collybia dryophila* and *Sclerotinia bataticola* to Hertzian waves of wave-length 50 and 100 m. for periods up to 65 hr.

Tverskoy (1937), using *Fusarium solani*, *Sclerotinia libertiana*, *Botrytis cinerea* and *Phytophthora infestans*, experimented with wave-lengths of 20 and 30 m. and 10·4 m. and less. In the shorter wave range, waves of 5·6 m. inhibited growth and killed the fungi more quickly than any other. Increasing the field strength shortened the time necessary to produce these effects. This was done by decreasing the distance between the condenser plates from 3 to 2·5 cm. Tverskoy found further that the higher the

temperature between the condenser plates the quicker were the inhibition and lethal effects produced. He noted significantly that *Fusarium solani* was killed in 5 min. in an electric field when the temperature of the medium reached 50° C., while the fungus had to be immersed in a water bath for 30 min. at the same temperature before it died.

Imshenetzki & Nazarova (1937) killed *Merulius lacrymans* and *Poria vaporaria* by exposing them in pure culture and in 5 cm.-thick wood blocks for 30 and 40–60 min. respectively. The wave-length employed was 4·5 m. With this wave-length *Merulius lacrymans* was killed in 50 min.: 8 m. waves took 60 min. to kill the fungus. If the field strength was much reduced neither fungus was killed even after 2 hr. The position of the wood blocks in the condenser did not affect the sterilizing action.

Metlitzky & Soboleva (1936) found wave-length critical and also showed a temperature effect. They used agar cultures of *Sclerotinia libertiana* and *Botrytis cinerea* placed between condenser plates 12 cm. in diameter in a secondary tank circuit. They obtained negative results with exposures from 1 sec. to 30 min. to a wave-length of 2·2 m. With 5·6 m. the fungi were killed in 20–25 sec. The medium began to melt when in the condenser at 45° C. but in a water-bath only at 80° C. Lethal effects began when the temperature of the medium in the condenser reached 30–40° C. These temperatures were not lethal under ordinary conditions.

Gier (1938) killed bacteria using 35 cm. waves. This seems to be the only record of the use of centimetre-length waves against plants. He observed no lethal effects with wave-lengths between 27 and 45 cm. on cultures of *Actinomyces scabies*, *Saccharomyces* spp., *Alternaria solani*, *Cephalosporium* sp., *Diplodia zeae* and *Gibberella saubinetti*.

The possibility that the effects produced are due purely to rise in temperature in a dry atmosphere is not excluded in any of the work quoted. On the contrary, it is difficult to see how fungi can be placed between the plates of a condenser without being exposed in a very dry atmosphere which would rapidly kill all hyphae unless protected from desiccation by the medium in which they were growing. The condenser method is open to the additional criticism that its practical application is limited by the fact that it would be awkward to apply on a large scale owing to the limitations imposed by condenser size. It seemed worth while therefore to experiment with very high frequency electromagnetic waves obtained from a source which made it possible to 'spray' wood-destroying fungi with the radiations from a directional and mobile aerial system. If lethal effects were obtained a practical method of controlling fungus attack in constructional timber, etc. would be made available.

Apparatus used

In the experiments now to be described, a standard Service radar transmitter was used as a source of radiation. This apparatus employs a cavity magnetron in conjunction with a pulse generator to transmit pulses of radiation of wave-length 9·1 cm., with a pulse length of the order of 1 μsec., pulse frequency of about

500/sec. and a peak power of approximately 55 kW. A concentrated beam of radiation is produced by means of a dipole aerial and a parabolic reflector.

DESCRIPTION OF EXPERIMENTS

Cultures of *Merulius lacrymans* and *Coniophora puteana* were used. Exposure times varied from 5 min. to 1 hr. and distances from the transmitting aerial from 9 to 41 cm. The aerial system was arranged to give a horizontal beam of radiation.

Glass slides. C. puteana was grown on very thin agar films on glass slides (Sass, 1929). After inoculation the films were grown for 4 days in damp chambers consisting of petri dishes lined with damp filter-paper. Growth was then vigorous and had spread about 1 cm. from the point of inoculation. The slides were clamped to a retort stand in a vertical row with the film side towards the aerial and 36·5 cm. from it. The transmitter was then run for 5 min. Control slides were similarly exposed in a dry atmosphere at the same room temperature. Both in the irradiated and in the control slides the superficial mycelium collapsed on the surface. All slides were then returned to their damp chambers. The mycelium on the irradiated slides did not grow any more. The hyphae looked sodden and developed a yellowish colour. The control films soon recovered and continued normal growth for a week. All films were then fixed in $\frac{1}{2}$-strength Flemming's fluid and stained with haematoxylin and light green (Gwynne-Vaughan & Barnes, 1937). Superficial hyphae tend to be distorted and vacuolated when treated by this method and this was found in both irradiated and control films. But normal hyphae in the surface of the medium were very few in number in the former and numerous in the latter. Growth in the irradiated slides was definitely checked but the fungus was not killed. The same type of adverse effect on growth is produced by exposing slides to dry conditions for a period of several hours or to a higher temperature for a shorter period.

Temperature effect of radiation. For the reason mentioned above attempts were made to detect a rise in temperature in the irradiated slides by placing a sensitive thermometer against the slides at the instant the transmitter was switched off. No increase was detected. As a further test, however, sloped tubes of 2% malt agar with and without mycelium of *C. puteana* were exposed for 30 min. Immediately afterwards thermometers reading room-temperature—19·75° C.—were thrust into the tubes so that they penetrated the surface of the medium. The reading on the thermometer in medium plus mycelium rose 5·65° C. while that in medium alone rose 4·85° C. A rise in temperature of this order would result in the drying of the thin layer of medium in an agar film. It gives an adequate explanation of the check to growth produced by irradiating films.

Glass tubes and plates. A number of experiments was carried out using thicker layers of medium in tubed malt agar slopes and poured plates. Distances from the aerial of 9·1–41 cm. were used and exposure times of 30 and 60 min. The medium was inoculated with mycelium of *C. puteana* or *Merulius lacrymans*. The cultures in the plates were exposed directly to the radiation by removing the petri dish tops and

wedging the plates in a vertical position; those in the tubes were exposed through the glass only. All tube cultures showed signs of 'sweating' by the end of the exposure. This did not occur in control tubes. In all the irradiated cultures the superficial mycelium differed from that of the controls in appearing damp and sodden. Irradiated cultures could be distinguished readily from controls for about 48 hr. after exposure: thereafter there was no visible difference. All irradiated and control cultures were transferred within 24 hr. of treatment. In every case the subcultures produced normal growth.

Wood blocks. There is a marked diminution in the intensity of the glow of a neon lamp, used as a rough indication of radiation intensity, when it is screened from the aerial by an agar plate during exposure. Thus the possibility existed that hyphae growing submerged in malt agar were being protected by the medium from the absorbed or reflected radiation, and that the growth obtained on subculturing was coming from such hyphae accidentally transferred with the superficial mycelium.

No diminution in the brightness of the neon glow was observed when the lamp was held behind pieces of wood up to 6 in. thick. The fungi were therefore inoculated on to beech and hawthorn wood blocks in boiling tubes and, after growth was well established, the blocks were irradiated at 13·65 cm. for 1 hr. either exposed directly or still inside the tubes. Transfers were then made to malt agar slopes from the front, side and back of the wood blocks. All transfers gave normal growth for the fungus concerned.

Conclusion

These experiments show that 9 cm. electromagnetic waves when used under conditions which eliminate the possibility of introducing large rises in temperature in a dry atmosphere—the conditions which exist if the condenser technique is employed—did not kill the mycelia of *Coniophora puteana* and *Merulius lacrymans*. Growth checks did occur when irradiation was accompanied by a rise in temperature of over 5° C. in a dry atmosphere. No attempt was made to estimate additional internal heating which might have occurred in the fungus mycelia.

The death of various fungi and bacteria, reported by other workers, is only to be expected if experimental conditions produce rises in temperature of the order of 20° C. in a dry atmosphere. It may well be that a temperature effect is the cause of all lethal action attributed to high frequency radiation in experiments using the condenser method.

I am indebted to Prof. Randle, late of the Department of Natural Philosophy, St Andrews University, for permission to use the radar transmitter and to Dr David Jack of the same Department for reading and commenting on this paper.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 21 March 1947, in the Imperial College of Science and Technology, London; the President, Mr W. C. Moore, in the Chair.

Symposium on biological problems of the leather industry

The following papers by members of the staff of the British Leather Manufacturers' Research Association* were read and discussed:

1. Introduction. By Sir Robert Pickard, F.R.S.
2. Some problems associated with collagen, reticulin and elastin in the skin. By Miss M. Dempsey and Miss M. E. Garrod.
3. Some aspects of the surface chemistry of animal skin. By Dr K. G. A. Pankhurst.
4. The microscope and leather manufacture. By Miss M. Dempsey. (Published in *J. int. Soc. Leath. Chem.* **31**, 317).
5. Influences modifying the growth and quality of animal skin. By Mr J. N. Turner.
6. Mycological and entomological problems of the leather industry. By Mr A. J. Musgrave.
7. Bacteria in leather manufacture. By Dr Margaret E. Robertson and Miss B. M. Haines.
8. Enzymes in the leather industry. By Dr E. W. Merry. (Not printed here).

SOME PROBLEMS ASSOCIATED WITH COLLAGEN, RETICULIN AND ELASTIN IN THE SKIN

By Mary Dempsey and Marjorie E. Garrod

The most important of the tanner's raw materials is the animal skin which he transforms into leather. The problems the skin presents to him are concerned with the types of tissues in the skin, their relative amounts and distribution in the skin and their arrangement in relation to one another as well as their chemical and physical properties.

The important tissues from a leather point of view are the fibrous tissues, collagen, elastin, reticulin and muscle. The epidermis and hairs are removed by the tanner.

Collagen is the chief component of the animal skin and it is to collagen that the properties of the leather, such as abrasive resistance, tensile strength, are due. The other fibres, though present in small amount, are of great importance in their effect on the collagen.

Collagen fibres in the skin are bound together into bundles by fine fibrils (Kaye, 1929), the nature of which is still something of an open question, but there are indications that it is reticular tissue.

* The authors are indebted to the Council of the Research Association for permission to read and publish these papers.

Elastin occurs for the most part in the grain and in the flesh layers of the skin, though there is also some elastin among the collagen fibre bundles in the corium.

Muscle fibres occur mainly as the *erector pili* and may be of importance to the tanner in their effect on the smoothness of the grain of the leather (McLaughlin, Highberger, O'Flaherty & Moore, 1929; Anderson, 1941, 1945) but muscle will not be further considered in this paper.

During the process of converting raw, putrescible skin into the more resistant leather two properties of the collagen fibres are of prime importance to the tanner, namely, their ability to combine with tan and their swelling properties. The collagen molecule is relatively unstable owing to the rather weak cross-links bridging the polypeptide chains of the molecule. Combination with tan results in the formation of stronger cross-links and confers an increased stability on the fibres as a whole.

The swelling properties (Jordan Lloyd & Shore, 1938) are brought into play at various stages in manufacture into leather. During liming the skin is treated with alkali for a number of days; subsequent acid treatment in the tan liquors involves a considerable lowering of the *p*H so that the fibres are subjected to wide changes in *p*H. The swelling of collagen at different *p*H values is therefore highly important and has received much attention. A considerable amount of the work has been carried out on rat-tail tendons, an easily obtainable source of relatively large collagen fibre bundles.

The results obtained with tendons are not necessarily entirely applicable to collagen fibres in the skin. In the first place, it is possible that the collagen molecule itself varies slightly according to the source, but the same general swelling properties are shown by collagen from all sources. Over the acid range there is a considerable increase in volume and a corresponding shortening in length with maximum effects about *p*H 2–2·5. There are similar changes on the alkaline side, although swelling is not so great.

In the second place, the properties of isolated fibre bundles are likely to differ from interweaving fibre bundles. In the skin a fibre bundle absorbing water must swell against the swelling pressure of its neighbours. Where fibre bundles are closely woven together, as in the butt part of the skin, swelling will be more restricted than where they are less closely woven, as in the belly region (Kaye & Jordan Lloyd, 1924).

In the third place, recent histological evidence suggests that the binding sheath in tendons is different from that in skin (Dempsey, Haines & Hitchborn, unpubl.).

The presence of the sheath around the skin fibre bundles is very important to the tanner because of its effect on the swelling and because it holds the fibres into bundles. The problems concerning the binding tissue are numerous. Its nature and structure need further investigation. It has been suggested that it is composed of reticulin (Kaye, 1929, 1936). A difficulty in studying the binding tissue in skin is to obtain a relatively pure sample. Reticulin prepared from fat deposits had a slightly different chemical composition from collagen (Jordan Lloyd & Garrod, unpubl.) and swelled less than collagen (Roddy & O'Flaherty, 1939).

Other problems relate to the amount and character of the binding tissue in the skin of different kinds of animals, in different parts of the same skin and in the same animal at different ages.

Jordan Lloyd & Marriott (1935) found young rat-tail tendons swelled less in acid than old, and Leplat (1935) found a big difference between the swelling in acid of cat- and kitten-tail tendons: are there similar age differences in the skin?

Elastin is readily demonstrated histologically in the skin but most of the work on elastin has so far been done on material isolated from *ligamentum nuchae*—the ligaments in the dorsal side of the neck.

The swelling of elastin is small and, in contrast to collagen, elastin fibres swell in all three dimensions (Jordan Lloyd, Dempsey & Garrod, 1946). This has been related to the differences in molecular structure of the collagen and elastin molecules. In collagen the long polymeric molecules are thought to be chiefly in the extended configuration, parallel to the fibre axis, so that swelling in length is almost impossible. The 'backbones' of the elastin molecules, on the other hand, probably lie in a crumpled and disordered configuration so that increase in length can readily take place. This molecular arrangement would also explain the rubber like properties of the elastin fibre.

Conclusion

In the skin, collagen is the most abundant of the fibrous proteins and its swelling and other properties are highly important in tannery processes. Elastin and reticulin, although present in much smaller amounts, are also of great importance. A study of their individual properties and their amount and distribution in skins is necessary. One of the difficulties up to the present has been to isolate samples of these proteins from the skin. Experiments on similar material from other sources, as e.g. collagen from rat-tail tendons, elastin from *ligamentum nuchae*, and reticulin from beef suet, have given valuable results, but they are not necessarily applicable even to the isolated skin proteins. In addition, the effect of the fibrous proteins each upon the other and the modifications of their individual properties when they are all woven together as in skin, must be known. Many problems still remain to be studied.

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SOME ASPECTS OF THE SURFACE CHEMISTRY OF ANIMAL SKIN

By Kenneth G. A. Pankhurst

(With 2 Text-figures)

The three main components of animal skin are: (1) protein (mainly fibrous, but some globular); (2) lipoids*; and (3) water. It is proposed to neglect such other components as carbohydrates, mineral salts, etc., which are usually present to a much smaller extent and, compared with the other three, are of less direct importance to the leather manufacturer.

During the conversion of animal skin to leather, changes are made in each of the main components:

I. The fibrous proteins are chemically modified by a tanning treatment.

II. The globular proteins are substantially removed.

* Throughout this paper the term 'lipoid' is used to indicate all the natural fatty material of skins, i.e. triglycerides, fatty acids, phospholipoids, cholesterol esters, etc.

III. The natural lipoids are usually removed at an early stage and are replaced by others practically at the end.

IV. The water content is considerably reduced.

The existence of the three phases, protein fibre—lipoid—water, gives rise to the possibility of six types of interface, each of which is of importance to the leather manufacturer at one stage or another of his processes, and presents its own peculiar problems. Especially is this so since the make-up of the fibre pattern in the skin gives rise to a collection of fairly long, capillary-like spaces of minute cross-section, through which various liquids may, or may not, be required to flow, both during and after the manufacture of leather.

We may summarize various operations which make up the manufacture of leather, according to their dependence on surface phenomena associated with these interfaces, thus:

Protein—lipoid. Degreasing, fat-liquoring, oiling, currying, etc.
Protein—water. Degreasing, tanning, waterproofing, dyeing, etc.
Protein—air. Curing, waterproofing, etc.
Lipoid—water. Degreasing, fat-liquoring, currying, etc.
Lipoid—air. Curing, oil tanning (chamoising), oiling (spues), etc.
Water—air. Drying, etc.

One of the attributes which has been largely responsible for the universal popularity of leather as a material for the manufacture of clothing (shoes, gloves, coats, and now even underwear) is its ability to allow the free passage of air and water vapour through its substance and at the same time to prevent the passage of liquid water. The combination of these properties in one material is made possible by the fibrous structure and its attendant *quasi* capillary spaces. The physics and chemistry of the flow of liquids through capillaries is therefore germane to this and many other similar problems in leather manufacture.

In the simplest case, the factors which determine whether or not a liquid will flow through a single capillary are (1) the work of adhesion between the liquid and the solid of which the capillary is composed, $W_{SL}=\gamma(1+\cos\theta)$, where γ is the surface tension of the liquid and θ is the contact angle which it makes with the solid, and (2) the cohesional work of the liquid, 2γ. Where the work of adhesion is equal to, or greater than, half the cohesion of the liquid, θ is 90° or less, $\cos\theta$ is positive, and the liquid flows through the capillary without the application of an external force. Where the work of adhesion is less than half the cohesion, θ is greater than 90°, $\cos\theta$ is negative, and the liquid does not flow through the capillary unless a minimum pressure, the 'penetration pressure', is applied. The magnitude of this pressure is dependent on the contact angle, surface tension, and radius of the capillary according to the following equation:

$$P=-2\gamma\cos\theta/r.$$

Although the same factors are important in determining whether or not a liquid will flow transversely across a piece of animal skin or leather, complications arise since the surface is not plane, but is gauze-like in structure. The fibres and interfibrillary channels which cause multidirectional corrugations on the surface are liable to cause considerable modifications in the effective contact angle, depending on the cross-sections of the fibres and spaces in the surface. Cassie & Baxter (1944) have shown that the farther apart are the fibres in the surface the greater is the effective contact angle. Fig. 1, taken from Cassie & Baxter, shows the change of contact angle brought about by the 'structure' of the surface, for various fibre diameters and interfibrillary spaces. It is thus possible for a liquid making a contact angle less than 90 in a single capillary, not to penetrate a piece of skin composed of the same material, the contact angle having been effectively raised to above 90° by the 'roughness' of the surface.

The penetration pressure, however, is of considerable use as an index in many problems involving the penetration of liquids at many stages in the leather-making process where effective contact angles greater than 90° are encountered, and is an easily measurable quantity (Edwards, 1931; Jovanovits, 1932; Stather & Herfeld, 1935; Robinson & Topp, 1942).

One of the earliest penetration problems which the tanner may have to face is that of soaking back skins which have been cured by drying in the direct sun. The excessive heat to which

such skins are often exposed causes the interfibrillary proteins, the albumins and globulins, to coagulate and cement the fibres together (Kaye & Jordan Lloyd, 1924). Obviously, penetration cannot take place into capillary spaces which are virtually non-existent, and means have to be adopted to peptize and remove these cementing materials in order to permit water to enter the system and the fibre structure to open up. Salts with polyvalent anions such as citrates and ferrocyanides (Jordan Lloyd & Pickard, 1923) have been used, as have also solutions of simple salts such as sodium chloride or nitrate or mild alkalis (Pleass, 1937).

It is customary with light skins, such as those from sheep and goats, to remove as much of the natural lipoids as possible at an early stage in the leather-manufacturing process, to prevent their interfering with later operations and imparting grease blemishes of one kind or another to the finished product (Innes, 1942; Davies & Innes, 1946). One method is to extract the lipoids from the damp skin with an organic solvent such as paraffin. This presents serious physico-chemical difficulties since paraffin makes a contact angle with water-containing skin of about 140°, and only penetrates when a pressure of about 15 cm. of mercury is applied to it.

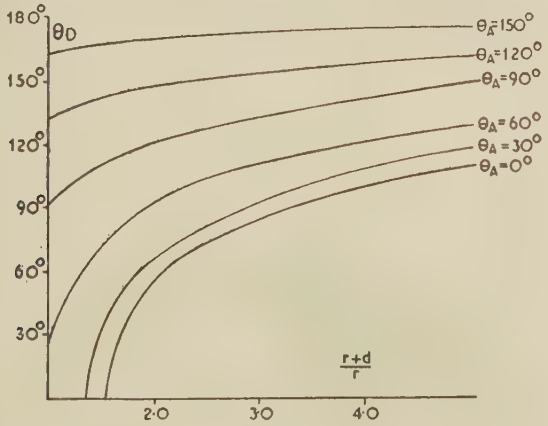

Fig. 1. The effect of 'structure' on the effective advancing contact angle. r = radius of the fibres; $2(r+d)$ = distance apart of fibre axes; $\frac{r+d}{r}$ is therefore a measure of the openness of the grid. (*From* Cassie & Baxter, 1944).

Means have to be found to increase the adhesion between protein fibre and paraffin relative to the fibre-water adhesion. This can be accomplished by the use of anionic wetting agents. The action of such compounds on proteins of the collagen type is such as to produce an oleophilic surface on the fibres, by electrostatic adsorption of the amphipathic anions to the charged amino groups of the basic side chains of the protein under acid conditions, and to the keto-imide backbone groups at higher *p*H values, by an ion-dipole adsorption mechanism (Pankhurst & Smith, 1945). Gelatin, in fact, can be thrown out of solution as an oil by the addition of an anionic paraffin-chain salt such as dodecyl sodium sulphate. Moreover, this oil can be dissolved in organic solvents such as toluene, once it has been dried (Pankhurst & Smith, 1944). The choice of anionic agent for aiding the penetration of an organic solvent into wet skin is, however, restricted by the fact that too great an increase in protein-solvent adhesion renders the removal of the solvent correspondingly difficult at the end of the process (Pankhurst, 1946).

Another method of removing grease, without the use of an organic solvent, is by aqueous emulsification. Here, again, the choice of agent is limited since either of the ionic types is

adsorbed to the protein under certain conditions of *p*H. Consequently, non-ionic emulsifiers of the ethylene oxide-alcohol (or phenol) condensate type are used (McLaren, 1947).

One important factor might be mentioned here, in connexion with degreasing skins. In the living animal, the skin lipoids are contained in fat cells, the walls of which have to be broken before any degreasing treatment can succeed. Balfe, Bowes, Innes & Pleass (1940) showed that if skins are given a pickle treatment (i.e., immersed in a solution of sulphuric acid and common salt), in which condition they contain relatively little water (*c.* 55 %) and are conveniently transported from one side of the world to the other, the subsequent period of storage is sufficient to bring about the rupture of many of the fat cell walls. With old animals, however, particularly sheep, the skins of which may contain as much lipoid as protein, many of the fat cell walls resist this treatment and remain intact throughout pickling and degreasing operations (Innes & Pankhurst, 1947). During drying after subsequent tanning, however, they are ruptured and the skin lipoids are able to ooze through the interfibrillary spaces and reach the surface of the finished leather. The search for a method of ensuring the complete rupture of all the fat cell walls prior to degreasing is one of the problems on which we are at present engaged.

Having removed natural lipoids at an early stage in the leather-making process, it is necessary to reintroduce a quantity of grease at the conclusion of the process, in order to maintain the splitting up of the fibrils of the leather and to provide a lubricant between them. Various oils may be used for this purpose, animal, mineral, or vegetable. It is, of course, important in introducing the grease, not to block up the interfibrillary spaces since this would destroy one of the most important properties of the leather, its ability to permit the passage of air and water vapour.

One method by which grease can be introduced, is in the form of a fine oil in water emulsion—the 'fatliquor'. Sulphated or sulphonated natural oils are commonly used as emulsifiers for this purpose, although the use of cationic (Somerville, 1932; Roth, 1940) and non-ionic (Clayton, 1935) agents has been described. One consequence of the use of anionic emulsifying agents is that the stability of the emulsion which they produce is governed by such factors as *p*H, and the salt content of the skins. Particularly are multivalent cations such as aluminium and chromium critical. Low *p*H values tend to reduce the stability of the emulsions since the hydrogen salts of the stabilizing anions are less dissociated and more easily hydrolysed than their neutral salts, and this reduces the net negative charge on the oil droplets of the emulsion. Polyvalent cations tend to desorb stabilizing anions from the oil-water interface with the production of insoluble salts. The protein itself adsorbs such ions, the extent to which it does so depending on *p*H (Pankhurst & Smith, 1944). It is, doubtless, this latter factor which gives rise to the characteristic unevenness of penetration of emulsified grease throughout the skin when applied in this manner; the emulsion being broken fairly soon after entering the skin, with the resulting deposition of dispersed grease near the surfaces. If the emulsifying agent is a mixture of sulphated oil and a quaternary nitrogen compound such as lecithin, the stability is altered and penetration is made more even between grain surface and interior as is shown in Fig. 2, which is taken from Merrill (1928).

The function of the anionic sulphated oil is mainly that of an emulsifier and not primarily that of a lubricant. It is the emulsified neutral oil that has this role, although it is likely that the adsorbed layer of ions of the sulphated oil does, by virtue of its rendering the fibre surfaces oleophilic, assist a little with the lubrication of the structure.

Oils may also be applied directly to damp leather; a process known as 'currying'. Marriott (1934) has shown that only when leather contains less than 68 % of water can oil be so introduced. Balfe & Uryash (1939) have studied the mechanism of the penetration of oils into damp leather and have shown that a low interfacial tension between the oil and the liquid with which the leather is in contact is of importance in assisting penetration. Low viscosity of the oil also increases the rate of penetration. The mechanism appears to be the spreading of the oil over the damp fibres. Triglyceride oils are more commonly used than pure mineral oils owing to their superior spreadibility, but mineral oils to which a surface active agent such as oleic acid is added can be just as effective as triglyceride oils (Balfe & Uryash, 1939).

• The introduction of grease into leather has an important bearing on its waterproofness. In particular, the formation of trivalent metallic soaps has been shown to be of no little importance in obtaining this desirable property (Pressley, 1946).

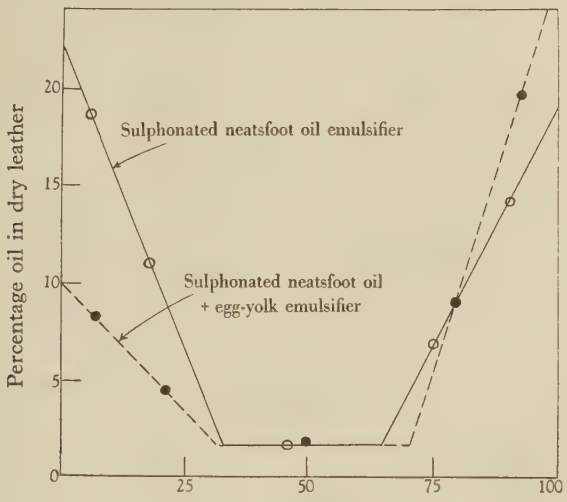

Fig. 2. The effect of lecithin on the distribution of oil throughout the thickness of chrome-tanned calf leather, during fat-liquoring. (*From* Merrill, 1928.)

In this review, it has been possible only to give the barest outlines of some of the many aspects of surface chemical research in which the British Leather Manufacturers' Research Association, and indeed, all leather chemists, are deeply interested. Many colloid problems have been omitted, including the extremely important theory of tanning and its many facets of surface chemical action (Jordan Lloyd, 1935*a*, *b*; Cheshire, 1943), and those wishing to study these matters further are referred to the following works: Wilson (1941); Jordan Lloyd (1943, 1946); McLaughlin & Theis (1945).

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INFLUENCES MODIFYING THE GROWTH AND QUALITY OF ANIMAL SKIN

By J. N. Turner

Differences in skin quality with their consequent effects upon the resulting leather have undoubtedly been recognized from the very earliest times. Until comparatively recently, however, little could be done to modify such effects; the tanner had simply to learn as best he could the differences between species, breeds and individuals. He discovered by trial and error how to modify his processes to suit the different types of skins and how to select for particular kinds of leather. The problems of selecting and processing have now become

complicated by the increase in varieties of skins and a disadvantage suffered by the modern tanner is that the quality of an animal's skin for leather manufacture is usually disregarded by the stock breeder, whose main concern is with meat, wool or milk. But, recently, as the importance of the leather industry has grown in certain countries, special attention has been paid to breeding for skin quality as rated by the tanner. In France and the Dominions, the problem of animal husbandry and its effect on the leather industry is treated as of serious moment.

It seems not improbable that many of the existing trends in breeding that are reacting to the disadvantage of the tanner may have to be modified to save certain sections of the leather industry from extinction.

Skin changes due to breed

Cattle. So many changes have been made in the last few decades in breeding—all with the aim of improving products other than the skin—that the leather trade is now suffering from an acute shortage of the more valuable types of hide and skin which were at one time plentiful.

In 1870 improved breeding was said to be producing thin, spready hides in the ox, so that the English tanner had to import his butt hides from Spain and Portugal. The same trend was expected to restrict the output of heavy hides to Texas and South America (Schultz, 1876). Among the results of the new methods of animal husbandry was a change from the desirable heavy, coarse hide typical of the draught animal to a finer, thinner hide. The efforts to produce breeds of dairy cattle with high milk yields have invariably led to the evolution of thinner skins (Zimmermann & Török, 1933). A characteristic of skins from high-yielding milch cows is the larger number of fibre bundles per unit area in the corium, and their smaller diameter in the reticular and papillary layers (Chiodi, 1933), although a comparison between the skin qualities of the various cattle breeds of northern Italy has shown no correlation between skin thickness and milk yield (Borgioli, 1935; Chiodi & Masi, 1933).

In England during the last 30 years, dairy cattle have increased from 8 to 24% of the total cattle, while the hardy, slow-growing breeds, most suitable as a source of heavy leather, have decreased from $5\frac{1}{2}$ to $1\frac{1}{2}$% (White, 1944). The dominant breed in England to-day, the dual purpose Shorthorn, while admirable as a producer of meat and milk, is a lowland type giving a flat leather suitable only for cow-hide and box-calf goods. Similar changes on the Continent, that is, an increase in lowland breeds at the expense of the hill cattle, are leading to a universal shortage of skins for heavy leather (Grassmann & Trupke, 1942). Environment modifies breed differences between mountain and lowland cattle. Mountain cattle have a thicker, coarser, and tougher skin than those living in the plains, and this has been variously attributed to their use as draught animals, to their improved nutrition arising from the greater availability of milk as a stock feed in the remote mountain farms (Huc, 1946), and to the colder climate and more exposed conditions (U.S. Dep. Agric., 1939). Thus, in similar areas of the skin, the European mountain Spotted has been found to be 22% thicker than the lowland Brown (Carstens & Kinzelbach, 1933), although other results have shown no significant difference between either these or other common breeds (Schotterer, 1933).

It is highly probable that some differences in the quality of leather alleged to exist between one country and another may be ascribed to the effect of breed. It has been claimed as the result of wear trials on the test track at Clairvaux in France, that French vegetable-tanned sole leather is greatly superior to similar leathers from England, Canada, or the United States (Gellé, 1946). A comparison between the distensibility of British, Continental, and American chrome calf leathers, in which the leathers were subjected to pressure over an area in the Lastometer, showed that the Continental leathers were more distensible than the British and American, which were approximately equal. They were also thicker, on the average, than the two other groups, and had fibre bundles which were fuller and had a greater compactness and angle of weave, the last being directly related to distensibility (Conabere, 1938). Unfortunately, no data were available as to the age of the calves at slaughter, and it is possible that the differences found were merely due to age.

In South Africa, the problem of animal breed and leather quality is being studied in great detail at the Leather Industries Research Institute. The first investigation to be made compared the quality of vegetable-tanned sole leather from Sussex, Afrikander, Red Poll and Friesian hides from herds brought up and fed under essentially the same conditions and slaughtered at the same average age. The heaviest and densest hides were obtained from the native Afrikander stock; these also gave the best sole leather as measured on the Thuau abrasion machine. The Friesian hides were the least dense and gave the poorest leather: spready, empty and uneven. Microscopic examination showed some relationship to exist between the compactness and angle of weave and the quality of the leather, as for the leathers examined for distensibility, mentioned above. The microstructure of the leathers was typical for each individual breed and with practice it was possible to identify the breed by examination of leather sections (Bisschop, Gardner, Sebba & Shuttleworth, 1943).

In a second study, a comparison was made of chrome-tanned upper leather from 1- and 2-year old Herefords and Shorthorns, pure-bred or crossed with Afrikanders, the selected animals being reared under identical conditions. The leathers were of excellent quality on the whole but in the semitropical climate the crosses with native stock possessed heavier skins and gave extremely good leathers, whereas leather from the Herefords was flanky and had a poor grain. Measurements of tensile strength, at shoulder and rump, showed that cross-breeding tended to reduce strength, although the number of samples was too small for conclusive results. The amount of stretch at break was also measured, but no certain indication as to the effect of breed was revealed, although it seemed likely that the pure-bred European cattle produced the more stretchy leathers (Gardner, Shuttleworth, Bonsma, Hirzel & Murray, 1943).

Sheep. Breeding developments in sheep are having an equally adverse effect upon leather quality, especially in the wool-producing countries, where the Merino is the paramount breed. In 1880, the Vermont Sheepbreeders' Association favoured the exaggerated development of skin folds in the Merino, whereby the total wool-bearing area was increased by an addition to the grain or epidermal layer, without a similar addition to the flesh layer. This gives a heavily ribbed skin which is unsuitable for the manufacture of leather. An adverse effect of the heavy skin folds is that the sheep are rendered highly susceptible to blowfly strike in Australia and South Africa (Carter, 1941). An effort is being made in South Africa to correlate differences in breed with quality of leather with the ultimate aim of evolving a breed which will produce good wool, meat and skins.

It has been found from preliminary work that Merino skins yield a leather which shows extreme ribbiness with a loose, coarse, pin-holed grain: Corriedale skins are somewhat thin with a smooth grain, and Persians have full skins with a tight, fine grain (Kritzinger, 1945). The excessive fat layer between grain and flesh which characterizes the Merino usually necessitates the skins being split before tanning. The best skins produced by crossbreeding the Merino came from crosses with the Dorset Horn, although the leather made from them was by no means of good quality (Kritzinger, 1946).

Other experiments on crossing the Merino have been made in Europe where wide breed variations exist (Diomidova, 1932) and where aboriginal stocks with little or no differentiation between the papillary and reticular layers of the corium are available for experiment. Promising results have been obtained, and it is stated that skin characters tend to be inherited from the ram (Teodoreanu, 1934; Serra-Efimova, 1938). The skins of long-woolled sheep are tougher, coarser, and thicker than those from the fine-woolled varieties, the fine-woolled mountain breeds having a delicate goat-like grain and a soft pelt free from excess of fat. The ancestral types in the countries of the Near East have a spready, thin, extra tough-grained skin which may be used for imitation glazed kid (Seymour-Jones, 1913).

Pigs. Few investigations concerning breed and skin quality of pigs have been made. It has been shown that, in two breeds at least, a relationship exists between distribution of the skin glands and hair follicles and breed, but there is no relationship between skin thickness and breed (Nicolau, 1939).

Nutritional factors affecting skin

Feeding can have a very important effect on the skin as is illustrated by a comparison of the cattle of West Africa with those of the same breed in Europe and by a comparison of the cattle of Australia with those of the smaller Pacific islands. Poorer nutrition gives a smaller animal with a thinner, finer skin (Schotterer, 1933). Cattle dead from starvation always show a fine texture of the hide (Schultz, 1876). The threefold difference in price between Paris calf boots and ordinary boots has been explained by a manufacturer as due to the fact that suckled calves give a leather of superior durability, appearance, and texture, which may last six times as long as a leather from bucket-fed calves, which is 'britchy'* and soon shows surface flaws and cracks (Picton, 1946).

Type of feeding is of considerable importance, especially to the tanner of sheepskins, since it may necessitate big changes in pre-tanning operations and may bring about serious defects in the leather. Nevertheless, despite its great importance, little scientific work has been done on the effects of differences in feed. One classic experiment has been the examination of the leathers produced from nine pairs of twin lambs, fully fed and underfed. The one group received a ration sufficient only to maintain weight, and the other received a normal fattening ration, so that after 16 weeks the members of this group had doubled their body weights. Average skin weight and skin thickness were almost double in the well-fed group and total area of the finished leather was 67 % higher. The differences in properties on an equal thickness basis were surprisingly small or non-existent, and the leather from the underfed lambs had less fat, a flatter, finer grain and a tighter weave, and was thus in many ways the better of the two (Clarke, Stuart & Frey, 1937). In fact, much of the trouble now being caused to the tanners of sheepskins arises from modern methods of intensified feeding. Furthermore, as fat in sheep is becoming one of its most valued products, methods of feeding are likely to be directed towards increasing it.

Intensive feeding causes a migration of body fat to the outlying tissues where much of it enters the skin (Seymour-Jones, 1909). In sheep's skin there is a fatty layer lying between the grain layer and the corium, especially marked in breeds such as the Merino and Lincoln. The size of this layer and the rate of its formation regulate the looseness of the skin and its value to the tanner. During storage, the fat cells of the skins may rupture; the fat then hydrolyses with later spue formation, an effect which becomes noticeable after liming owing to the formation of insoluble lime soaps. (Innes, 1941.)

An experiment was carried out in America to show the effect of artificial feeding upon grease in heavy leathers after attention had been drawn to the fact that hides from certain stockyards which stall-fed their cattle with cotton-seed before slaughter possessed a very yellow fat deposit along the backbone which later showed up as a grease stain on the finished leather (Oberlander, 1931). Hides were examined from yearling steers given three different diets, one a normal high grain fattening ration, the second restricted in concentrates, and the third also restricted but containing much cotton-seed. The first group possessed the most greasy hides; the second group had no grease stains. There was no direct relationship between grease content of the hides and fat content or net energy value of the feed, and it was assumed that individual differences must outweigh the effect of feeding. A second experiment with a limited number of steers given rations providing high and moderate energy values and digestible fat contents produced more conclusive results, all hides of the better-fed group giving greasy leather while those of the other group gave leather with no grease marks. With allowance for slight individual variations it was shown that the weight increments of the animals were related to the fat content of the hides. Whenever the fat content in the hide exceeded 12 %, grease stains appeared on the leather. Moreover, microscopic examination of greasy hides revealed that fat deposition occurs at the expense of the hide substance, producing a loose, open fibre weave and a leather which is lacking in firmness and body, particularly after degreasing (Frey, Clarke & Stuart, 1933).

* Soft and spongy.

Initial steps in the study of metabolism of skin fat include investigations of the lipid distribution in horizontal layers of the skin and the effect of diet on the deposition of triglycerides (Koppenhoefer, 1936; Lustig & Perutz, 1932; Haldi & Wynn, 1941, 1942). Studies in the distribution of fat in skins have already led to the formulation of a more scientific process for degreasing (Innes & Pankhurst, 1946).

Age, sex and environmental differences

While the differences in leather quality due to sex are generally well known, the modifying effect of age on these differences is not so widely appreciated. Larger animals generally have thicker hides, but thickness apparently decreases with age in some if not all portions of the hide (Carstens & Kinzelbach, 1933). Bulls have thicker hides than cows but give a softer, less resilient leather which is liable to tear more easily (U.S. Dep. Agric., 1939). It is claimed that cow collagen contains more histidine and less arginine than bull collagen (Tadokoro, 1933). With increasing age the bull's skin becomes coarser while the cow's becomes finer, and although the absolute thickness of the epidermis does not change with age, the relative thickness increases in cows and decreases in bulls (Schotterer, 1933). Rams have a thicker, firmer skin than ewes and wethers (U.S. Dep. Agric., 1939); the fat content of the skin tends to increase with age (Seymour-Jones, 1909).

Tensile strengths of the finished leathers increase with the age of the animal though variations are very wide, depending upon the choice of sampling position and method of tanning: statistical treatment of the problem has not yet been attempted (Jordan Lloyd, 1943).

Climatic conditions affect skin quality as shown by comparison of hides from Canada and Russia with those of the same breed from the United States. A warm climate produces a fine texture; a cold climate results in a coarse texture. Within even a few months, seasonal climatic conditions affect skins, and hides from animals killed at different seasons receive their appropriate grading. Cattle hides are thin in spring and grow thickest in September and October, in preparation for the winter, when the growth of hair causes the hide temporarily to lose its firmness (Schultz, 1876). Seasonal variations in lamb-skins are very marked and of particular importance to the tanner, the differences being largely due to the size and condition of the sweat glands which function to the greatest extent as the growth of wool increases in the warm weather. The leather produced from summer skins is consequently spongy and loose. When there is an early spring the lambs mature more quickly and are slaughtered before the hot weather, to give a superior leather (White & Caughley, 1936).

In the adult sheep the season of growth influences the quality of the leather, this being largely associated with fat metabolism. Rapidly growing wool absorbs all existing fat stocks except the cholesterol type fats which are increased by the action of sunlight (Roffo, 1935), and are associated with 'cockle' disease. This is skin eruption, commonly occurring during the spring, caused by the sebaceous glands becoming swollen by a cholesterol fat which the wool is not able to take up and which appears as a waxy exudation on the skin (Procter, 1912). A pimply skin results when deposits accumulate at the bases of the hair follicles. Most severe cases of cockle arise when oilcake and a dry feed form the basal diet (Seymour-Jones, 1913). The effect of cholesterol content on susceptibility to insect attack might be worthy of study, since it is known that hide beetles require a diet rich in cholesterol (Hinton, 1945).

Differences induced by artificial means

Skin quality may be influenced by artificial methods which may be of no practical importance but which may, nevertheless, throw light upon the course of skin metabolism and the differences which arise in nature.

Repeated administration of oestrogens, for example, will bring about a decrease in skin thickness, both of epidermis and dermis, with a reduction in the size and number of the sebaceous glands and hair follicles (Hooker & Pfeiffer, 1943; Arguello, 1937). Skin thickness has been increased in mice by daily inunction with dibenzanthracene, cystine disulphoxide,

or thiocresol, the last, at a concentration of 0·5 % in petrolatum, causing a threefold increase (Reimann & Chatalbach, 1937). The modulus of elasticity of skin has been increased by subcutaneous injection of silicic acid or by soaking pieces of skin in silicic acid solution (Kochmann & Maier, 1930). It is significant that grass contains considerable amounts of silica, and that the level of silica content is a varietal character.

Conclusion

It is certain that the quality of the animal skin is of great importance to the tanner, and it seems that modern trends in breeding and feeding of domestic animals are militating against the eventual production of certain important classes of leather, though the extent to which this is appreciated outside the industry is doubtful. Although, in normal times, the tanner can exert some control by discrimination in purchase, major improvements can best come from effective propaganda, possibly with the co-operation of the Hides and Allied Trades Improvement Society.

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MYCOLOGICAL AND ENTOMOLOGICAL PROBLEMS OF THE LEATHER INDUSTRY

By A. J. Musgrave

Mycological problems

The fungi associated with the leather industry may all be grouped under the headings of moulds and yeasts. Mostly they are harmful to the industry, though use has been made of some of them. Briefly, we may say that various fungi will grow on certain finished leathers, on the raw hides and the various liquors and on the skins during processing; they may cause stains, hydrolyse triglyceride fats and cause spues (which are unsightly patches of exudate); or they may grow on the greases applied in currying. Fungi also grow on the living animal, where they are sometimes pathogenic.

Yeasts are found chiefly on the vegetable tan-liquors. Chambard & Seux (1939) claim that certain yeast-like forms may serve a useful function in contributing to the necessary acidification of the tan-liquors. That yeasts are not entirely harmless, however, is shown by Doherty's (1939) statement that some pink spots which occurred on white chrome leather were due to yeasts. It appears, too, that such intermediate forms as *Monilia variabilis* may do damage (Chambard & Belperron, 1945) in vegetable tan-liquors by destroying tannin.

But it is, I think, the organisms commonly referred to as moulds (or sometimes mildews) which create most of the industry's mycological problems. Undoubtedly the two most commonly mentioned groups are those of *Aspergillus niger* and *A. glaucus*; I suspect that this is because they are relatively easily recognized: and this leads me to say that the identification of the moulds associated with the industry is one of our problems.

I want now to outline the problems created by the mould group of fungi. We know something of the impairment of leather-making properties done to a living skin by pathogenic fungi—there is a paper by Stuart & Frey (1935) on the subject. As regards the problems arising during processing, we know, from the work of Blank (1932) and Pleass (1936), that prolonged growth of moulds on pickled skins does cause damage. (During pickling skins are subjected to the action of an acid and a salt mixture.) Recently, Dohogne & Thomas (1946) have investigated some pickled sheepskins that were badly blemished by black spots: after some trouble they were able to isolate the organism as of an *Oidium* type. Moulds are very likely to occur on some vegetable tan-liquors, where they are liable to destroy the tan and cause the production of non-tans; some species are said also to produce darkly coloured substances that stain pelt tanned in the liquor (Anon., 1926). In general the astringent catechol tans support mould growth less well than the pyrogallol tans (Jordan Lloyd, 1934, quoting Robertson). Some moulds produce tannases: enzymes that bring about the breakdown of tans (Prescott & Dunn, 1940). The tannases would seem to be specific, for Nierenstein (1922) has shown that a catechol tannase is produced by *A. luchuensis* when grown on a catechol tan, and a pyrogallol tannase is produced when it is grown on a pyrogallol tan. Mould growth is also associated to some extent with the precipitation of water-insoluble ellagic acid (McLaughlin & Theis, 1945). Apart from these disadvantages, it is very likely that skins tanned in mouldy liquors will yield leather more liable to mould. Moulds grow more readily on the weak tan-liquors that have been used for some time than on the fresh liquors.

After tanning in either vegetable or chrome liquors, skins that are left about wet form a good substrate for moulds (Blank, 1933). Very frequently such processes as currying and fat-liquoring in which the tanned skin is treated with oils or greases or oil emulsions leave the leather very liable to mould growth in a damp atmosphere. I have found in the laboratory, that leather treated with a moderate amount of oil may form a better substrate for mould growth than the unoiled leather. The oils by themselves seem, however, to be a very poor

substrate. The action that moulds may have on oils and greases in the leather is very complicated: they may cause damage by hydrolysing greases of the triglyceride type and result in the formation of solid fatty spues (Jordan Lloyd, 1934); on the other hand, Balfe (1937) has shown that certain oils, such as cod oil, in leather undergo a spontaneous oxidation and produce a gummy spue. This is prevented, however, by the presence of fatty acids that may be liberated from the triglyceride greases by moulds. None the less, the presence of fatty acids in the leather should be controlled, as they cause further complications if the leather is brought into contact with certain metals.

Moulds will grow on finished leather, whether chrome or vegetable-tanned, though they grow much more easily on the vegetable-tanned. The extent of the growth depends upon the processing and finishing that the leather has received. Apart from the obvious damage that they do to the appearance of the leather, and this kind of damage may be bad for commercial prestige, moulds may cause a loss of tensile strength in the leather (Evans & Critchfield, 1933; Kanagy, Charles, Abrams & Tener, 1946), or they may cause stains. Mouldy leather may also act as a focus of infection (Smith, 1945): for example, mould growth on a leather case may spread to valuable apparatus or instruments it contains. Furthermore, Groom & Panisset (1933) suggested that the droplets of water secreted by *Penicillium chrysogenum*, growing on bookbinding materials, including leather, might help the spread of mouldiness in a library.

The extent to which mould hyphae penetrate into leather substance is obviously of importance when considering the amount of damage done, and we know that the mycelium can be detected well inside the leather structure. Two histological techniques have helped to this end—one by Raymond (1937) and a more recent one by Rose (1946) employing pyronin methylene green. Neither technique is perfect, and it may be remarked that this detection of mould hyphae in the matrix of leather is another of the minor problems of the research side of the industry.

We do not know exactly what is the food of the fungi that grow on leather; it may be the water solubles, as is suggested by the work of Robertson (1934), or the various finishes that are applied to leather. Vegetable-tanned leather is sometimes treated with magnesium sulphate, and this may aid mould growth, for Morris (1927) has found that magnesium sulphate and chloride sometimes nullify the toxic action of zinc chloride, and Galloway (1935) has shown that, in the presence of an adequate food supply, magnesium salts (the chloride and sulphate) exert a marked stimulant effect on fungal growth.

Before concluding this section on mycology, mention must be made of the use of fungi in the service of industry. Enzymes produced from moulds have been used, according to Kaverzneva & Oleinikova (1932) and Grimes (1946) for freeing wool from skins with minimum damage to both wool and skin.

Thus our mycological problems can be summarized as the prevention of fungus growth on skins, tan-liquors, greases and finished leather; the identification of the various fungi; the detection of mycelium in leather; the fuller study of the physiology of the relevant fungi to enable us the better to effect their control, the better to learn the extent of the damage they may do, or, in some cases, the better to harness them in the service of industry. Finally, a knowledge of the fungus diseases of the living animal and of how they affect the leather-making qualities of the skin would no doubt help the tanner to detect diseased skins before processing, thus enabling him to reject these skins or modify his works procedure so as to get the best results from this poor raw material.

Entomological problems

The entomological problems of the industry may appear at first sight to be few; but insects cause extensive damage to hides and skins while they are still part of the living animal; they cause serious damage to dried hides, particularly in hot climates; they are, at least, a nuisance on hides and skins undergoing wet processing; they cause damage to furs; and they damage

leather. Table 1, which gives the results of a careful survey of the literature, shows that a considerable number of insect species are at least associated with the industry. As with other commodities, the damage done to leather and the raw materials of the industry may be either direct damage, where the insect uses the material as a food, or indirect damage, where the insect uses the material for pupation or tunnelling, etc.

Insect pests of the living animal certainly create problems for the industry. Their remedy lies outside its direct control, but through the Hide and Allied Trades Improvement Society efforts are made to improve the quality of the raw material. The two common warble flies do an immense amount of damage (O'Flaherty & McLaughlin, 1930; Hearle, 1938) to hides by puncturing them or causing the development of scar tissue; their larvae spend a period of their lives encysted under the skin of the backs of cattle, and subsequently emerge through a hole in the skin. One animal may have many warbles, as the encysted maggots are called, and the resulting holes lower the value and usefulness of the skin. The sheep tick (*Ixodes ricinus*) is notorious in the agricultural world as a vector of diseases in cattle and sheep. An apparently new problem caused by it came to light about a year ago, when I was shown some sheepskins that were spoiled by a number of holes. The skins had been partly processed, but it was possible to find associated with one hole a tick, identified by Mr T. Spence, of the Veterinary Laboratory at Weybridge, as *I. ricinus*. An interesting feature of the particular hole was the collar of skin around it.

Though demodectic mange and scabies damage skins (Dempsey & Robertson, 1937), we do not know if psoroptic mange does.

An unusual form of damage is that reported by Bergmann, Hausam & Liebscher (1932) as due to lice, *Trichodectes scalaris*: the damage appears to have been extensive, and the authors suggest that the lice do more damage than is often thought.

On dried hides the chief pests are beetles of the genus *Dermestes*. Damage done has been estimated, according to Hinton (1945), in tens of thousands of pounds and is greater in hot than in temperate climates: the beetles are certainly a major pest. Four species of Lepidoptera are also reported from dried hides. On hides and skins undergoing wet processing, and particularly on wet-salted skins, the cheese skipper is fairly common; it is attracted by salt and has a great nuisance value in that the adult fly is very 'fussy', and the infested tannery comes under criticism as a focus of fly breeding. It is, of course, not unlikely that the larvae and the flies spread putrefactive bacteria. Recently I found the larvae of an Anisopid on some wet skins (Musgrave, 1946*a*); I do not know if they were doing any damage.

Clothes moths and carpet beetles are notorious pests of furs. Mallis (1945), quoting Kemper, states that rabbit, calf, goat and wild-cat furs are subject to slight damage by clothes moth larvae; but skunk, marmot and opossum are severely damaged. It seems that larvae cannot complete their development on certain skins, e.g. rabbit and calf. The larvae consume the hide at the base of the hair, thus causing it to fall out (Mallis).

As regards finished leather, the extent of the damage is variable. Chrome-tanned leather appears not to be attacked. Termites damage leather in the tropics. Leather may be seriously damaged by *Ptinus tectus* (Musgrave, 1946*a*; Stather, 1932), and from personal observation I suspect that migrating larvae of Lepidoptera may do damage to leather in their search for a pupation site. *Dermestes* may make numerous burrows in heavy leather (Line, 1934); *Anthrenus verbasci* and *Sitodrepa panicea* have been reported damaging leather-bound books; and *Anthrenus pimpinellae* has been recorded damaging saddlery in Egypt.

We may sum up our entomological problems as being chiefly:

(i) The protection of the living skin from gross defects caused by insect attack. This is only an indirect problem in the leather industry, and one that it can tackle only by propaganda.

(ii) The protection of hides and skins awaiting manufacture into leather: that is, while they are in store or piled up.

(iii) The treatment of finished leather and furs with insecticides as a protection from insect attack: one instance where this is particularly desirable is the proofing of clothing and upholstery leathers for export to warm climates or for use on ships.

TABLE 1. *Insects and other arthropods associated with the leather industry.*

Order of insects	Location of pest: The living animal	Hides and skins	Furs	Leather
Anoplura (lice)	*Trichodectes scalaris*, Nitsch	—	—	—
Isoptera (termites)	—	—	—	Termites
Coleoptera (beetles)		*Dermestes maculatus* Deg.	*Ptinus tectus* Boield.	*Anthrenus vorax* Waterh.
		D. lardarius L.	*Attagenus piceus* Oliv.	*A. verbasci* L.
		D. oblongus Solier	*Anthrenus scrophulariae*	*A. pimpinellae* Fab.
		D. frischii Kugel.	*A. museorum* (L.)	*Attagenus byturoides* Sols
		D. cadaverinus Fab.	—	*Niptus hololeucus*
		Necrobia rufipes	—	—
			Trogoderma ornatum (Say)	*Sitodrepa panicea*
		Attagenus piceus (Oliv.)	—	*Anthrenus scrophulariae* L.
		Sitodrepa panicea	—	*Dermestes maculatus* Deg.
				Trogoderma versicolor (Creutz.)
				Lassioderma serricorne
Diptera (flies)	*Hypoderma lineata* de Vill.	*Piophila casei*	—	—
	H. bovis Deg.	*Prodesmonetopa latipes* Mg.	—	—
	Melophagus ovinus L.	Unidentified Anisopid larva	—	—
	Glossina morsitans Wstw.	—	—	—
Lepidoptera (butterflies and moths)		*Monopis monachella*	*Tineola biselliella* Hum.	*Tinea fuscipunctella* Haw.
		Tinea pallescentella Stainton	*Tinea pellionella* L.	*Setomorpha margalaestriata*
		Borkhausenia pseudospretella	*Trichophaga tapetzella*	*Borkhausenia pseudospretella*
		Trichophaga percna Corbet & Tams	*Tinea flavescentella* Haw.	—
Other arthropods	*Demodex*	—	—	—
	Sarcoptes scabei	—	—	—
	Psoroptes sp.	—	—	—
	Ixodes ricinus L.	—	—	—

The following were consulted in the compilation of the table: Bergmann, Hausam & Liebscher (1932), Corbet & Tams (1943), Griswold & Greenwald (1941), Hearle (1938), Hinton (1945), Line (1934), Mallis (1945), Musgrave (1946*a*), O'Flaherty & Roddy (1932), Patton (1922), Solodovnikova (1938), Stather (1931, 1932).

It should be noted that only where an authority for the species was available in the work consulted has it been given in the table. The species given were reported associated with the product named. For full details as to damage, etc., the original work should be consulted.

These problems can be considered under two heads: problems of laboratory testing and problems of incorporation during manufacture.

Laboratory problems. There is a difference of approach to the testing of insecticides from that adopted for fungicides, for in the one case one is usually dealing with an active, moving animal, and in the other with a small sedentary plant. The difference is best seen by considering that in the laboratory the method in testing insecticides is to treat insects and observe subsequent mortality, whereas in testing fungicides the method is to treat a substrate and observe the extent of mould growth with time in an atmosphere of optimum conditions.

As regards the laboratory testing of insecticides, the problems in this industry are not very different from those in any other. Consideration has to be given to the type of carrier used to dissolve the toxic material; to the type of insecticide that the industry will find most advantageous, i.e. whether it should have a contact, a stomach or a residual effect, or a mixed effect; to the species of insect most commonly occurring as a pest that is easily bred in a laboratory; and to the way in which the insecticide is to be applied, e.g. as a spray or powder or fumigant to hides, as a mist or film in the tannery, or as an impregnant to finished leather. As all these variations existed it was decided in our own work to use *Dermestes maculatus* as the test insect, and to start by trying to compare insecticides having a residual effect, using filter-paper as a substrate to be impregnated by the toxic materials and subsequently walked on by the test insect.

This method has given promise of being quite effective, although it is at present in its early stages of elaboration. So far D.D.T. and Gammexane have been incorporated in the tests; and we have found Gammexane very much more toxic than D.D.T. to *D. maculatus* in preliminary experiments (Musgrave, Turner & Rose, 1947).*

The laboratory testing of fungicides presents many of the difficulties encountered in insecticide testing; in addition there is the added difficulty of sterilizing leather, though O'Flaherty (1946) has very recently described a rather elaborate technique. For this reason we have thought the inoculation of treated samples in most cases unnecessary. For our standard tests we use unsterilized vegetable-tanned leather as the substrate, and we find that untreated samples always grow mould readily in a damp atmosphere. Of course, this means that we are, so to speak, at the mercy of those species of mould fungus that become predominant on any test sample. But so far the technique has withstood reasonably well the demands made upon it. Undoubtedly it will have to be improved if we are to make close comparisons between similar fungicides. Leather itself is a variable material, and it may well be that we shall have to use a strictly standard tannage and type of skin for our tests; that is to say, something more nearly standard than we are at present using.

Another problem is the method of treatment of the laboratory experimental leather: should it be sprayed or painted with fungicide, should it be dipped in the fungicide or should the fungicide be applied to it by some dropping method? After some trials we have favoured the dropping method, as we are then sure of the amount of toxic material on the leather. For solvents we have favoured alcohol and neatsfoot oil, which is one of the oils used in the trade.

A considerable amount of attention has been devoted to laboratory tests on fungicides for the industry. Shuttleworth (1940) tested a number of compounds, using a specially modified tan-liquor solution as a substrate. He found that the *p*H of the solution affected the fungistatic properties, and that phenyl mercury acetate was better than *p*-nitrophenol. Robertson (1934) has also used tan-liquors as substrates in fungicidal tests: she used an inoculum consisting of *Aspergillus niger*, *A. ochraceous*, *Penicillium glaucum*, and *P. luteum*. Lollar

* Cf. Bovingdon, H. H. S. & Stock, G. H. (1947). *J. int. Soc. Leath. Chem.* **31**, 115.

(1944*a b*) has tested a great number of fungicides in the laboratory, using leather as a substrate. He used a number of solvents and a method of application that seems to have given him little control over the actual dose of toxic material on the leather. Furthermore, after the application of the fungicide, the samples were subjected to washing in water. The tests thus took the form of screening tests conducted on tannery lines in a laboratory, rather than precise toxicological experiments. They have, of course, considerable practical value for this reason. Tests by Morrow & Richardson (1940) employed phenol derivatives accurately applied to wet leather that was subsequently inoculated with mixed moulds and subjected to 100% humidity atmosphere. In preliminary experiments two compounds protected for 16 days, which was the longest period: they were sodium pentachlorophenate and chloro-2-nitrophenol.

Recently the Physical Testing Committee of the American Leather Chemists' Association (Anon., 1945) have evolved a detailed provisional method for testing the resistance of leather to fungal growth. It is based on conventional lines: pieces of leather to be tested are compared with a standard mould-susceptible leather; the leathers are given a fairly rigorous wash in water; they are then inoculated with a standard inoculum and incubated at appropriate temperature and humidity. The inoculum consists of a fungus spore mixture in sand prepared by the Tanners' Council Laboratory of the University of Cincinnati.

Manufacturing problems. Apart from the problem of the warble fly, which is really an agricultural or veterinary problem, the insecticidal problem that has received most attention on the field or industrial scale is the control of the attack of *Dermestes* beetles of hides and skins. Much work has been done in South Africa, and nowadays arsenication is practised very widely. Hides and skins may be dipped in or sprayed with the appropriate solution. According to Furlong (1937) a suitable solution can be made from white arsenic, water and caustic soda: he gives two methods of preparation. Alternatively commercial sodium arsenite can be used. The strength of the solutions used and the time of immersion vary in practice. Smit (1934), for example, recommended the use of a $2\frac{1}{2}$% solution immediately after flaying; this was ten times the strength of solution used commercially (Furlong, 1937).

Recently Kritzinger (1946) has favoured the use of sodium fluosilicate; he recommends dipping for 15 min. in a saturated solution, and claims that the leather made from salted hides treated in this way is up to standard. These treatments are favoured because they are cheap and easy to apply. Hides and skins are usually treated before shipment, as, it seems, damage may otherwise be extensive. The main problem here has been to find a treatment that is cheap and does not interfere with the subsequent processes of leather manufacture. As much of this treatment must be done by African and Indian labourers of very varying educational standards any method must be relatively simple.

Very little has been done on the control of flies or cheese-skippers on wet hides.

Although a vast amount of work has been done on the moth-proofing of woollen fabrics, little seems to have been done on furs and wool sheepskins. Among the interesting moth-proofing methods that have been elaborated, and that could doubtless be made applicable to furs, it seems worth mentioning the famous 'Eulan' series of compounds (Hartley, Elsworth & Barrit, 1943), the elaboration of modified wool resistant to the reducing enzymes in the gut of the moth larva (Geiger, Kobayashi & Harris, 1942), and an attempt at proofing furs by Traill, McLean & Bovingdon (1944).

With regard to the proofing of finished leather for use in the tropics, our Research Association has carried out some preliminary experiments in co-operation with the Ministry of Supply, the Indian Army Ordnance Department* and two member firms. The experiments were designed to ascertain if it were possible to protect leather from insect attack by treating it with D.D.T. The test insects were termites and *Anthrenus vorax*. Two interesting points have so far emerged from the results: first, that D.D.T. may be used successfully to protect leather from *Anthrenus*, and secondly, that though termites in India may seriously damage

* Inspectorate of General Stores, Cawnpore (M.G.O. branch), G.H.Q., India.

untreated leather, in West Africa the damage done is only slight. These results are still being co-ordinated, and it seems unwise to make any further statement about them here.

The chief problems associated with the practical application of fungicides in the industry are now concerned with the choice of the appropriate fungicide and the investigation of new ones. The classical material of the industry is 'anti-gallic', a mixture of *o*-, *m*- and *p*-cresols. During the last ten years this has been joined by *p*-nitrophenol and β-naphthol. Dempsey (1945) has, fairly recently, summarized the methods whereby fungicides may be incorporated into leather: they may be added in the late tan-liquors, in a special dip, in the fat-liquoring oils or in the stuffing grease; or finished leather may be sprayed with an alcoholic solution of a fungicide. So varied are the kinds of leather and the uses to which they are put, however, that almost every proofing problem merits separate attention. As regards mouldy tan-liquors, it will be appreciated from what has already been said that the kind of tan and the *p*H of the liquor are important factors. Pelts in pickle are liable to go mouldy, and this may be prevented by the use of *p*-nitrophenol or by *p*H adjustment by the use of acetic or formic acid (see Musgrave, 1946*b*, quoting Pleass & Blank).

A great number of fungicides have been tested in the past, and more are constantly appearing. It may be of interest that we are just now experimenting with *p*-nitrophenol, β-naphthol, various phenyl mercury compounds and chlorinated phenols. It should be added that every one of the fungicides at present available to the industry has disadvantages of one kind or another.

When deciding which fungicide or insecticide to use, it is important to know whether the leather to be treated is to be used for clothing, upholstery, panelling, textile machinery, shoe soles, washers, luggage, or machine belting, as each one of these uses raises its own separate problem.

Finally, in all these problems, the health hazard to the industrial worker and to the purchaser of the finished article, by the use of the fungicide concerned, has to be considered, and must eventually be carefully checked.

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BACTERIA IN LEATHER MANUFACTURE

By M. E. Robertson and B. M. Haines

Throughout the long process of converting raw skin into leather constant precautions have to be taken against bacteria, especially the proteolytic bacteria which convert collagen into gelatin as the first stage of putrefaction, then slowly liquify it.

The quality of the finished leather depends to a large extent upon the condition of the raw material and the majority of the defects in hides and skins reaching the tanneries are due to bacterial action.

It has been shown by Tissier & Martelly (1902) that the aerobic types of bacteria initiate the putrefactive processes and by creating a favourable medium in the tissues encourage the

proliferation of the anaerobes which then take up and carry on the degenerative changes. The cellular structures such as sweat glands, fibroblasts and the basal layer of the epidermis are the first to be attacked, then the interfibrillary proteins, and finally the collagen fibre bundles.

The changes taking place in a freshly flayed piece of ox hide during a period of 8 days after its removal from the animal have been followed macroscopically and microscopically at the British Leather Manufacturers' Research Association laboratory (Kaye, 1927). There were no obvious macroscopical changes till after the fourth day, when bad odour, slimy feel, and the beginning of hair slip indicated that putrefaction was in progress. Microscopically, however, the first signs of putrefactive change, commencing with shrinkage and degeneration of the cellular structures, were observed as early as 3 hr. after death. The collagen fibre bundles, 11 hr. after flaying, had become thinner, more separated into fibres, and less closely packed. After 2 days, when to all outward appearances the sample was still fresh and in good condition, microscopical examination showed that the grain was losing its cellular structures and becoming thin and compressed, and that the collagen fibre bundles of the corium were still thinner, more loosely packed and more horizontally woven. Some of the changes described, especially the very early ones, are more probably due to autolytic enzymes generated in the hide itself, than to bacterial enzymes, since no great proliferation of bacteria would take place in 5 hr. Raised temperature greatly favours autolysis as it of course favours bacterial growth and attack. Moisture also encourages bacterial growth in general, and lack of air the growth of anaerobic bacteria. To maintain hides and skins in good condition, therefore, they should be stored in cool, dry conditions with abundant air supply.

The method of entry of bacteria into the hide has also been studied in the B.L.M.R.A. laboratory (Robertson, 1932*b*). It has been shown to take place almost entirely from the flesh surface until putrefactive changes are well advanced; the keratinous layers being apparently too tough for bacterial penetration. Dempsey has shown that as putrefaction of the hide proceeds the reticulin binding tissue round the fibre bundles attacked is broken down and the fibres and fibrils forming the bundles become free to spread out and separate, giving rise ultimately to soft, loosely woven, unsatisfactory leather (Dempsey, 1940).

Under the heading 'putrefactive changes' should be mentioned also the loosening of the hair by bacterial action, a process known to sheepskin tanners as 'sweating', and still made use of, especially in Australia, in the treatment of merino sheepskins. Maxwell & Lennox (1944) have described work on this subject: out of seventy-five species of bacteria identified on sheepskins only four apparently possessed wool-loosening activity, the chief among them being *Proteus vulgaris*.

The ideal raw material for the tanner is the raw skin immediately after flaying and cooling. Since this is an ideal seldom or never attainable, hides and skins for leather making must be put as quickly as possible into a condition to resist bacterial and autolytic decomposition, i.e. be cured.

Bacterial growth on animal tissues can be inhibited by depriving such tissues of moisture (drying), or by keeping them at a low temperature (cold storage). They can be killed or their growth inhibited by the use of substances directly toxic to them (antiseptics) and/or osmotically unfavourable to their development (sodium chloride brines of high concentration); or they may be held in check by a combination of dehydration of their nutrient environment with direct osmotic or toxic action on themselves (dry salting).

Drying is the oldest and simplest method of cure, and has the advantage of reducing the weight of the hides or skins and so making their transport easier and less expensive. When drying is done directly in the sun it is likely that the bactericidal as well as the dehydrating power of the rays of the sun play a part in the cure. There are, however, disadvantages connected with this method of cure: (1) A tendency towards what the tanner calls blistering, i.e. sloughing of patches of the grain during tanning. (2) A tendency towards general putrefactive changes where drying has been unusually slow, as in a tropical rainy season. (3) Greater difficulty than with wet-salted hides in soaking back to something approaching their original condition. (4) The very special attraction dried hides—especially those that have been badly

fleshed and in which putrefactive changes may have begun—seem to possess for some species of beetles. Many hides arrive in this country badly 'worm eaten', as it is called, by hide beetles.

Salting with sodium chloride is by far the most usual method of cure other than drying. In dry salting inhibition of bacterial growth is due mainly to dehydration of the hide or skin tissues. In wet salting or brining inhibition of bacterial growth would appear to be due to osmotic changes in the bacteria themselves brought about by the brine, for which effect very high concentrations of salt are needed, or to some directly toxic action of salt on the bacterial plasma, or to both causes. The inhibitive action of sodium chloride can be enhanced by the addition of other chemical substances such as sodium carbonate, which probably acts by creating a *p*H too alkaline for bacterial growth, or sodium fluoride which would appear to be directly toxic to many bacteria, especially those producing the slimy red growth often seen on hides cured with salt of marine origin and known to the tanners as 'red heat'. Curing with sodium chloride has also disadvantages and among them are: (1) The cost of the sodium chloride. (2) The weight of the salted (especially the wet-salted) hides as compared with the sun-dried. (3) The occurrence of stains due to impurities in the salt or to inefficient methods of salting. (4) The fact that in very humid climates, as in India during the monsoon, sodium chloride deliquesces and goods salted with it become wet. The brine formed by the solution of the sodium chloride may then drain away leaving the goods open to bacterial attack. Sodium sulphate, the principal ingredient of the Khari salt much used for curing in India, remains dry and exerts an inhibitory action on bacterial growth.

The moist red growth known to the tanners as 'red heat' has been mentioned above. This is produced by the proliferation of halophilic or 'salt-loving' bacteria found in salt prepared by the evaporation of the sea water (Robertson, 1931*a*, 1932*a*). Ordinary bacteria of dirt and dust either die or lose their power of reproduction when subjected for more than a day or two to concentrations of salt above 8%. They can, however, be gradually 'conditioned' to withstand higher concentrations. Halophilic bacteria, on the other hand, multiply best at concentrations of 25–30%, and do not grow at all at concentrations below 6%. There are various forms of halophilic bacteria; those most commonly found are large cocci producing red growths and multiplying like sarcinae, by dividing into fours and so producing little packets. Some of the red growths are dry, some wet and slimy. Some liquefy gelatin, others do not. They are found mostly on the flesh side of hides and skins, but may also grow on the hair side and down into the hair follicles, and where growth has been heavy and infection long standing they can eat into and cause serious damage to the surface of the hides and skins. The addition to the curing salt of sodium fluoride or sodium silico-fluoride in a 1% concentration on the weight of the salt inhibits the growth of halophilic organisms (Robertson, Marriott & Humphreys, 1934).

Soaking. To remove dirt, to bring dried hides and skins back to something like their original state of moisture and to remove salt from salted ones, they are soaked in water before going on to the next stage of the pre-tanning processes—liming. Unless firmly dealt with, bacteria may increase enormously in the soaks and irreparable damage be done. Ideal conditions, especially in the summertime, of moisture and warmth are provided for the great numbers of them that are brought in with the goods, and they multiply very rapidly, finding their food material mainly in the interfibrillary proteins of the skin. If they would stop at these, which have to be removed in any case, their presence would be welcomed by the tanner—indeed, the use of putrid soaks was at one time an established tannery process. But having used up the interfibrillary proteins they go on to attack the collagen fibres themselves, especially in goods where taint has occurred previous to soaking. Slight damage is accentuated and leather yield reduced and greater damage may be so increased as to render the goods unusable. Light leathers, in particular, are liable to be spoiled in the soaks. So much of their substance may be eaten away as to render them thin and papery, the grain surface may be pitted or even destroyed over considerable areas or so weakened that it cracks easily on flexing or stretching. The use of a disinfectant in the soaks is therefore advocated

by the B.L.M.R.A. and chlorine in one form or other has till now been found the most effective and the most practicable. It is not necessary or perhaps even desirable, since unhairing is rendered more difficult, to sterilize the soaks completely. It is enough to keep the numbers of bacteria down to reasonable proportions (Jordan Lloyd, 1935).

Liming. It comes as something of a surprise to the bacteriologist to find bacteria surviving immersion in a saturated lime solution, together with a small proportion of sodium sulphide, for a period of 9–10 days; in the form of spores, nevertheless, certain bacteria do (Collett, 1921, 1922, 1924). The high alkalinity of the lime liquors destroys actively growing bacilli and most cocci, but work at the B.L.M.R.A. laboratories has shown that the spores produced by some of the bacilli survive throughout liming and become active again when conditions are favourable to them. This is the case with the anthrax bacillus which has always been a problem in the leather industry. Even anthrax spores can, however, be destroyed by a 'warm liming' process in which a fairly high proportion of sodium sulphide is used in the saturated lime liquor. The liquors are heated at the beginning of each day and allowed to cool gradually and the goods are drummed gently at intervals throughout the liming process. In these conditions the goods can be sterilized in about 5 days (Jordan Lloyd & Robertson, 1930; Robertson, 1931*b*).

Bating, puering and drenching. Before going on into the acid tan-liquors some at least of the alkali in the limed pelts must be removed. This can most easily be done by neutralizing with a weak acid, and, for heavy hides, where the first of the tan-liquors of itself does most of the neutralization and only a surface deliming to ensure good colour in the resultant leather is required, this is the method usually employed. But for light leather, where smoothness, silkiness, and a flaccid, fallen condition of the grain are highly important, deliming is often a part of the bating process which may be defined as an enzyme action affecting the skin proteins that can only take place after the skin has been brought to a suitable *p*H—generally about 8.

It is now customary to use artificial synthetic bates made up of proteolytic enzymes absorbed on a carrier and containing ammonium salts mainly as deliming agents, but puers which are natural manures, such as dog or pigeon dung, containing the live bacteria producing the necessary enzymes, are still used. In puering processes, therefore, bacteria are of use to the tanner. The goods, however, remain only a short time—say 1–2 hr.—in bates or puers, and the process must be very carefully watched and stopped before it goes too far. The enzymes they produce cleanse the skin of interfibrillary proteins and any collagen that has been partly degraded by the lime. A recent theory of the action of puers and bates for producing a smooth silky grain is that they attack the *erector pili* muscles (the small slips of involuntary muscle running from the grain surface to the side of the hair follicles and by their contraction producing 'goose-flesh'), so weakening their attachment to the follicle wall that any contraction of the muscle that may subsequently occur, such as at the transference of the skin from the warm bate liquor to cold water, will no longer pull up the follicle and roughen the skin (Anderson, 1941, 1945). Many tanners still consider that a silkier grain is produced by natural than by artificial bates. In puers the active enzymes are present on solid particles fine enough to pass down the hair follicles to the attachment of the erector pili muscle: in artificial bates the sawdust or woodmeal particles on which the enzymes are adsorbed are too coarse to do this (Jordan Lloyd, 1946).

Fermenting bran drenches are used in some forms of light leather manufacture to clean and slightly plump the skin after bating and puering. The bacteria in these should be of the lactic- or acetic-acid producing type which do not attack the skin fibres. That contamination with collagen-destructive bacteria can occur in these drenches and do great harm, was well illustrated by a works problem presented to the B.L.M.R.A. by a sheepskin tanner. The trouble complained of was that his leather was coming through poor and empty and thin. Bacteriological examination of the bran drench showed the presence of a very active gelatine-liquefying organism and cleansing and disinfection of the drench paddles soon put matters right.

Tanning. In the first tan-liquors any lime remaining in the limed pelts is gradually

neutralized and a change from alkaline to acid conditions brought about. During this process there will be stages in which *p*H conditions become favourable to the growth of bacteria. The concentration of tan is too weak in these early liquors to inhibit bacterial growth, hence viable spore forms brought in from the limes may become active again and a potential source of damage to the goods.

The tan-liquors themselves are also likely to be harbouring micro-organisms from the materials used in preparing them, and from the air and dust and dirt.

Fermentations often take place in tan-liquors due to the activities of lactic-acid-forming bacteria and acetic-acid-forming yeasts (Blank, 1935; Doelger, 1935, 1936*a*, *b*, 1937). The bacteria grow more actively in the weaker liquors (*p*H range 4·0–6·6), yeasts in the stronger (*p*H 3·2–5·0). The nitrogen required for the support of these micro-organisms is supplied in practice by the hides.

Both fermentations go on better with a reduced rather than an abundant supply of oxygen, and some tan-liquors produce more acid than others because they contain more fermentable material.

Not much tannin destruction is caused by the fermentative bacteria or yeasts separately, but in combination they can produce a good deal: bacteria alone 4%; yeasts alone 8%; combined 30% (Doherty, 1937).

Lactic acid destroyers—bacteria which utilize lactic acid as a source of needed carbon—may also flourish in tan-liquors as well as the lactic acid producers. Hence, this gain in lactic acid may also be lost by bacterial activity. In some American tanneries these tan-liquor fermentations are utilized to produce some at least of the acids necessary to keep the tan-liquors at the desired *p*H. In this country, however, we consider them too difficult to control and play for safety by adding our acids out of the bottle, as it were, in the quantities required.

The above review has shown some of the work that has been done on bacteriology in leather manufacture. Much yet remains to be done in identification of the bacteria causing damage to hides, skins and leather and in devising methods of frustrating their attacks.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Conversazione on research methods and equipment held on Friday, 25 April 1947, in the Department of Botany, Imperial College of Science and Technology, London.

List of Exhibits

Anti-locust Research Centre

Aircraft spraying experiments against settled locusts. — Dr Gunn

Cambridge Botany School

Flagella of bacterial plant pathogens stained by the silver method. — Dr Dowson
Liquefaction of pectate gels by soft-rot bacteria and other organisms. — Mr Rudd Jones
Interaction between strains of *Trichoderma viride* and *Fomes annosus*. — Mr Rishbeth
Mastigosporium leaf spots of grasses. — Mr Bollard
Control of culture mites by cigarette-paper barriers. — Prof. Brooks

Corstorphine Seed Testing Station

Seed-borne diseases of economic crops. — Miss Noble

East Malling Research Station

Aerial photography in disease surveys.
Verticillium wilt of hops: field and laboratory studies. — Dr Keyworth
Separation of virus mixtures by aphis vector.
Grafting techniques for plant-virus investigations. — Dr Prentice
Field recording of fruit-tree red spider populations. — Dr Massee

Forest Products Research Laboratory, Princes Risborough

Effect of pruning on quality of wood. — Mr Scott
Deterioration of glues in plywood. — Dr Findlay
Laboratory rearing of stocks of wood-boring insects: the use of β-naphthol to control the predaceous mite *Pediculoides ventricosus*. — Dr Fisher and Mr Harris

Institute of Agricultural Parasitology

Plants parasitized by nematodes. — Dr Goodey

London School of Hygiene and Tropical Medicine

Control of scrub typhus in Burma. — Lt-Col. Audy and Mr Cockings
Antimalarial surveys in Syria. — Major Leeson
Respiration and inhalation of dust particles. — Mr Davies
Apparatus for seeing in the dark. — Dr Muirhead-Thomson

Long Ashton Research Station

Plant-tissue test technique for determining mineral deficiences. — Mr Nicholas
High-pressure spraying equipment: standardization of components. — Dr Kearns

National Agricultural Advisory Service: East Midland Province

Woodmouse attack on winter cereals. — Mr Roebuck

N.A.A.S.: South-west subcentre

Use of the Staniland drawing apparatus to record plant pathological symptoms. DR THOMAS

Pest Infestation Laboratory, Slough

Vacuum desiccator for determining the water content of single wheat grains. MR OXLEY

Subepidermal mycelium in dry wheat grains. MR OXLEY and MRS MILNTHORPE

Tests of wrapping papers for resistance to insect penetration. MR OXLEY and MISS ROOT

Use of cobalt thiocyanate papers to determine relative humidity. MR SOLOMON

Culture chambers for single mites. MR CUNNINGTON and MR SOLOMON

Method of studying sites of oviposition by *Tineola biselliella*. MRS BASDEN

Method for confining insects on vertical surfaces. DR PARKIN and MR HEWLETT

Plant Pathology Laboratory, Harpenden

Technique of testing resistance of potato varieties to wart disease. DR SMITH

New British plant diseases. MR MOORE

Reading University Department of Agricultural Botany

Mould fungi causing deterioration of stored feeding-stuffs. DR SNOW

Rothamsted Experimental Station

Multiple-point inoculating needle.

Age and susceptibility of potatoes to *Helicobasidium purpureum*. MR GARRETT

Eyespot of wheat in relation to plant density. MISS GLYNNE and MISS MOORE

Electron microscopy of plant viruses. MR CROOK and MISS SHEFFIELD

Ultra-high-speed photography. DR GREGORY (with assistance of Kodak Laboratories)

Adhesive trap for aphides. MR BROADBENT

Soil micro-organisms. MR JONES and MR SINGH

Slugs. DR BARNES

Relative abundance of species of insects. DR WILLIAMS

Graphical device for estimating developmental periods of insects. DR PRADHAN

Recent developments in experimental design. DR ANSCOMBE

Royal Holloway College

Potato tubers showing haustoria of *Phytophthora infestans* within the cells, and cork cambium isolating blighted tissues. MISS BLACKWELL

Royal Horticultural Society: Wisley Laboratory

Factors affecting insect populations.

Incidence of populations of social wasps (*Vespula* spp.).

Incidence of crop attacks by carrot fly (*Psila rosae*) and by the tarnished plant bug (*Lygus pratensis*).

Sexual ratios among colonies of earwig (*Forficula auricularia*) and of the large bulb fly (*Merodon equestris*). MR FOX-WILSON

A number of the leading scientific instrument makers exhibited recently designed equipment and apparatus.

During the afternoon the following films were shown in the Botany Lecture Theatre:

Vegetable insects. *National Film Board of Canada.*

The thistle. *Gaumont-British.*

Ultra-high-speed photography. *Kodak Laboratories.*

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1946

The Officers and Council of the Association were as follows:

President: C. B. Williams, M.A., Sc.D.

Vice-Presidents: Prof. W. Brown, M.A., D.Sc., F.R.S.; G. V. B. Herford, O.B.E., B.A., M.Sc.

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Hon. Editor of The Annals of Applied Biology: R. W. Marsh, M.A.

Hon. Asst. Editor: I. Thomas, M.Sc., Ph.D.

Council: L. A. Allen, Ph.D., D.Sc., F.R.I.C.; G. H. Bates, D.Sc.; J. R. Busvine, Ph.D.; M. Cohen, M.Sc., Ph.D.; H. J. Craufurd-Benson, B.Sc., Ph.D.; J. A. Freeman, Ph.D.; Miss M. D. Glynne, D.Sc.; Prof. F. G. Gregory, D.Sc., F.R.S.; G. V. B. Herford, O.B.E., B.A., M.Sc.; J. T. Martin, Ph.D., D.Sc., F.R.I.C.; R. T. Pearl, B.Sc.; F. J. D. Thomas, B.Sc.

The numerical advance of the Association in 1946 far exceeds that in any previous year and the present position is stronger than ever before. During the year 53 new members were elected and 2 old members rejoined. The Association records with regret the death of Dr M. Nierenstein, and 13 other members resigned. On 31 December 1946 the total membership, including 11 Honorary Members, was 436, 41 more than a year earlier. The Association was very happy to offer its congratulations to Sir John Fryer, a former President and Treasurer, on the conferment of his knighthood.

Five meetings held in London attracted large audiences, the average attendance recorded being 44 members and 35 visitors. On 14 June over 80 members and friends enjoyed the privilege of a visit to the University Horticultural Station and the National Institute for Research in Dairying at Shinfield, Reading. A day full of interest to all present concluded with tea provided by the kindness of the National Institute.

The following papers were presented to the Association during the year:

22 Feb. Discussion of the Official Scheme for the approval of proprietary products for the control of plant pests and diseases. H. Martin: Past attempts to establish an approval scheme. J. T. Martin: The structure and operation of the current scheme. J. R. Booer: A manufacturer's comments on the approval scheme. W. A. R. Dillon Weston: The approval scheme as seen by a specialist advisory officer. O. G. Dorey: The grower's impressions of the approval scheme.

12 Apr. Joint meeting with the Microbiological Panel of the Food Group of the Society of Chemical Industry. Problems associated with the growing and treatment of flax. G. O. Searle: Research and the flax industry. Prof. A. E. Muskett and J. Colhoun: The diseases of flax, with special reference to seed-borne diseases and their control. J. W. Brandon and C. B. Taylor: Recent developments in the retting of flax and the dispersal of waste waters. L. A. Allen: Recent developments in the microbiology of retting.

4 Oct. Symposium on the significance of trace elements in plants and animals. Sir Edward Salisbury: General Introduction. Prof. T. Wallace: Visual diagnosis of mineral deficiencies in plants. D. J. D. Nicholas: Chemical tissue tests. W. A. Roach: The use of leaf analysis, plant injection and curative treatment for the determination of mineral deficiency in plants. R. Hill: Trace elements in relation to enzymes. H. H. Green: Trace elements in relation to health of live stock.

8 Nov. S. A. Barnett: Rodent control in towns.

6 Dec. A. R. Wilson, A. E. W. Boyd, J. G. Mitchell and W. S. Greaves: Potato haulm destruction with special reference to the use of tar acid compounds. G. E. Blackman: Recent developments in chemical methods of weed control.

The Association is greatly indebted to the authorities of the Imperial College of Science and Technology and of the London School of Hygiene and Tropical Medicine for the provision of accommodation for meetings. Under the prevailing conditions lecture rooms have sometimes been made available only at considerable inconvenience to the Colleges concerned. These difficulties are likely to increase for some time to come and the indulgence of members is sought in respect of those occasions when lecture theatres available are not entirely adequate.

At the end of 1945 the Biology War Committee ended its activities and the Council has been thanked for the support given to the work of this Committee. The Council has assisted in the preparations for the International Congress for Microbiology to be held in Copenhagen in July 1947. Several members of the Association hope to attend and to contribute papers. The Council has also made representations to the Prime Minister on the subject of the extended use of Dartmoor for the purpose of military training.

PLANT PESTS AND DISEASES COMMITTEE, REPORT FOR 1946

Three meetings of the Committee have been held during the session, on 11 April and 7 November 1946, and 17 January 1947. G. L. Hey, C. J. Hickman, F. G. W. Jones and R. W. Sidwell were appointed by the Council to fill the vacancies caused by the retirements under the rules of Miss M. T. Franklin, G. C. Ainsworth, C. Potter and J. H. Western. At the first meeting J. T. Martin and W. G. Keyworth were re-elected Chairman and Secretary respectively.

The memorandum prepared by the Committee on the recruitment and training of plant pathologists in Great Britain has appeared in the *Annals* (**33**, 119) and has attracted much interest.

The Sub-Committee, formed in collaboration with the Ministry of Agriculture Conference of Advisory Entomologists, has made progress in the compilation of the list of common names of British insect and other pests. The first section, dealing with slugs and snails; plant parasitic eelworms; beetles; flies; sawflies, wasps and related insects, has been published in booklet form and may be obtained from I. Thomas, 47 Tennyson Road, Harpenden; free to members of the Association; price to non-members, 2*s*. net. The Sub-Committee is now working on a further section on plant bugs, aphides, etc., butterflies and moths, and the minor Orders.

At the request of the Plant Pathology Committee of the British Mycological Society, suggestions were made on definitions of certain terms used in plant pathology. The Committee has also dealt with a number of matters raised by members of the Association or by outside organizations concerning the study and practice of plant pathology.

HON. EDITOR'S REPORT FOR 1946

Vol. **33** of *The Annals of Applied Biology* comprised pp. viii + 465 and 13 Plates as against pp. viii + 380 and 12 Plates for Vol. **32**. Including papers and abstracts published in *Proceedings*, vol. **33** contained 69 communications, 60 being by members of the Association. The subjects may be roughly classified as follows: general applied botany, 7; myclogy and fungus diseases, 15; bacteriology and bacterial diseases, 1; viruses and virus diseases, 3; general zoology, 1; entomology and insect pests, 15; helminthology and nematode diseases, 2; applied microbiology, 2; insecticides and fungicides, 18; general, technique, etc., 5.

Publication continued to be delayed by printing and binding difficulties and the several numbers of vol. **33** were issued on the following dates: No. 1, 4 June 1946; No. 2, 8 August 1946; No. 3, 15 November 1946; No. 4, 10 March 1947.

During the year, the increased demand for the *Annals* necessitated the raising of the printing order from 800, first to 900, and later to 1000 copies.

While an increased allocation of paper has permitted a return to a more readable format for Vol. **34** of the *Annals* this does not imply a lessening of the need for conciseness in the presentation of papers. Any material addition to the total number of words printed in an issue will lead to further delay in production. Authors are therefore still required to condense their texts and to include only such tables and figures as are indispensable. Preference will be given to papers by members.

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDED 31 DECEMBER 1946

During the year ended 31 December 1946, the amount received for subscriptions and entrance fees, including arrears, was £533. 4*s*. 6*d*., which was £71. 6*s*. 0*d*. more than in 1945, and more than double the amount received in 1939. There were eleven subscriptions unpaid at the end of the year.

Income from the sale of the current volume of *The Annals of Applied Biology* amounted to £1077. 1*s*. 6*d*., an increase of £201. 19*s*. 0*d*. on the year and of over £400 on the 1939 figure. During the year it became necessary to raise the printing order from 800 to 900 and later to 1000 copies. The volume contained 465 pages compared with 380 pages in 1945, and the cost of producing it rose by nearly £600, to £1969. 17*s*. 10*d*. It will be recalled that in the Treasurer's statement last year it was mentioned that Council had sanctioned considerable additional expenditure on the *Annals* in 1946 in an endeavour to reduce delay in publication. There was again a tremendous demand for back numbers and parts, which realized £347. 1*s*. 5*d*.: a source of income that cannot continue at this level because the stocks of many volumes are already very low. There was an increase of over £50 in the sale of reprints, but despite these two rather exceptional items the balance against the *Annals* on the year's working rose from £173. 17*s*. 9*d*. to £455. 8*s*. 11*d*.

Income for the year exceeded expenditure by £37. 17*s*. 10*d*. In addition the Association received from the Royal Society a grant of £175 in aid of publication, which was acknowledged with gratitude. After all obligations had been met, the assets of the Association at the end of the year amounted to £1731. 13*s*. 4*d*., of which £775. 14*s*. 10*d*. was represented by National Savings Certificates and £96. 9*s*. 0*d*. by the estimated value of the stock of the *Annals*.

REVIEWS

Molds, Yeasts and Actinomycetes. By C. E. Skinner, C. W. Emmons and H. M. Tsuchiya. Pp. xiv + 409, with 136 text-figures and 5 tables in the text. New York: John Wiley and Sons. 1947. $5.

The traditional approach to the study of mycology has been through botany, but in 1930 *Molds, Yeasts and Actinomycetes* opened a new road, leading from bacteriology. In that first edition, as in the present issue, emphasis was naturally placed on the fungi growing for, or on, mankind. The study of these fungi has made such signal progress since 1930 that a second edition has necessitated a complete rewriting of the text. This task, started by the late Dr Henrici, has now been discharged by Drs Skinner, Emmons and Tsuchiya.

The introductory chapter, on structure and classification of the fungi, has been prepared by Dr Skinner from Dr Henrici's notes; the succeeding chapter, a judicious summing-up of the problems of variation in the lower fungi, is Henrici's own. Dr Skinner's chapter on laboratory culture methods follows. In the next ten chapters, Dr Skinner deals with the systematics, Dr Emmons with the medical aspects and Dr Tsuchiya with the biological activities of Phycomycetes, certain Ascomycetes, Fungi Imperfecti, Yeasts and Actinomycetes successively. A final chapter by Dr Tsuchiya treats of fungal antibiotics.

In the past development of industrial and medical mycology much has to be credited to the non-mycologists, but they also must be debited with formidable additions to taxonomic confusion. Dr Skinner deals faithfully with this problem and suggests means for repairing the damage done, for example, by the medical maltreatment of the genus *Monilia*. In his chapter on the yeasts and their allies he adopts the classification of Stelling-Decker for *Saccharomyces* and the Endomycetales, and that of Lodder for the asporogenous yeasts. He presents a new key to the Actinomycetales, adapted from that of Waksman and Henrici.

The chapters by Dr Emmons make up 120 pages and form an outstanding section of the work. The literature of the mycoses abounds in unproved hypotheses and dubious etiology in which the facts have become embedded. Dr Emmons has taken this embedded material, sectioned, cleared and displayed it to furnish a lucid picture of medical mycology. These chapters alone would justify the purchase of the book.

By comparison with the medical aspects, industrial mycology is briefly treated, on lines familiar in text-books of microbiology. A detailed account and diagram of the recent hypotheses on alcoholic fermentation is given: other useful tables list the acids, pigments, antibiotics and miscellaneous metabolic products of fungi. The allocation of only six pages of text to antibiotics is indeed exiguous, but explicable by non-availability of information during the war years in which the manuscript was prepared.

Molds, Yeasts and Actinomycetes, unique in providing a conspectus of the part played by these groups in medicine and industry, is indispensable to the microbiologist. But it can equally be commended to those concerned chiefly with the outdoor fungi. The rural mycologist reading this book would gain an increased respect for the capabilities of the fungi and would learn at least one consoling fact—that the chewing of straws is not (despite popular belief) a sure method of acquiring actinomycosis!

R. W. MARSH

Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve. By D. J. FINNEY. With a foreword by F. TATTERSFIELD. Pp. xiii+256. Cambridge University Press. 1947. 18*s*.

To most biologists the revolution in statistics, which we owe to R. A. Fisher, is associated with the publication of his *Statistical Methods for Research Workers* in 1925. This volume presented for the first time a comprehensive account of the methods which were to do so much towards replacing, by ready acceptance, the distrust which had been felt of statistical analysis. Yet in emphasizing the importance of these new methods, we should never forget that the revolution went even deeper. It was at root a revolution of principles; and Fisher's new principles provided the basis, not merely for his own developments, but also for those of the world-wide school of statisticians which has followed him.

Each branch of biology demands its own particular applications of these principles in the design of its experiments and the analysis of its data. The requirements of agronomy have led to the development of field plot technique and the associated analysis of variance. Though used elsewhere, these methods will always retain a special association with agronomical science. In the same way, the analysis of χ^2 and the theory of information have developed in particular relation to genetics. Now Mr Finney brings together the special statistical methods associated with toxicology and bioassay where the responses are quantal—methods to whose development, as Dr Tattersfield points out in the foreword, the author has himself contributed much.

The problems raised by quantal response are not confined to bioassay and toxicology. They have long been familiar in psychophysics, yet it will come as a surprise to many biologists to learn that their statistical treatment was understood in broad outline by psychophysicists some thirty years ago. The method has had to be rediscovered several times, the last being when, in the hands of Gaddum and Bliss, it began a dozen years ago to develop into its present form of probit analysis, with all the power and refinement that Fisher's principles could give. The wheel has now come full circle, for it appears that just as the earlier work of the psychophysicists escaped the biologists, with the consequence that they had to reinvent the analysis, so the improvements that the biologists have made are at present largely unknown to the psychophysicists!

The method of probit analysis is remarkable in both its underlying principle and its statistical practice. The percentages of individuals, from a test sample, responding or failing to respond to a treatment is related in sigmoid fashion to the dose. The first problem is to straighten out this line. To do so the frequency distribution of tolerances of individuals to the treatment is assumed to be normal. A percentage response can then be replaced by a corresponding tolerance level, which cuts off a fraction of the population of the size observed to respond, and whose value can be measured as a departure from the mean tolerance expressed in terms of the distribution's standard deviation. In order to avoid minus signs, five is added to this normal deviate and a probit value is obtained. The probit value is thus itself a measure of dose, but a measure expressed in terms of the standard deviation of the tolerance distribution whose value is unknown. The problem is then to graduate this probit measure in terms of the conventional measure of dose. When put this way, we can see that the probit value should show a straight line relation to any transformation (usually the log) of the conventional measure of dose which would give a normal distribution of tolerances. Finney demonstrates this theoretical basis of the probit method by the more rigorous, but (to the biologist) less easily comprehended, comparison of certain fairly formidable formulae.

The calculation of the straight regression line relating probit to dose, or log dose, would present no special features, if it were not for the different weights to be placed on the observations. These vary with the expected probit values, and so cannot be obtained until a regression line is available. The vicious circle is broken by drawing a provisional line by

eye, and using the weights it gives in the calculation of a better one. This improved line may itself be used as the basis of a still better approximation, and the process repeated as often as is necessary. The number of repetitions needed depends on the skill used in drawing a first line which approximates well to the true regression; and indeed, as Finney points out, the line drawn by eye, if well judged, will itself serve the purpose of most routine analyses. We have therefore the unusual case of a graphical method which is not merely an end in itself, but the necessary start of its own refinement by calculation. The process of fitting the regression line, calculating its error variances, and using it to compare the relative potencies of drugs, are all described with a wealth of illustrative example. The treatment of the fiducial limits to the line, to the ED 50 (which, where the response is lethal, becomes the LD 50) and to the measures of relative potency, is unusually informative and should go far to ensure that these limits are put to the best use in future.

Finney then moves on to consider further extensions of the method; extensions in which, one feels, he has had a special interest. He first shows us how to make the proper adjustment for natural mortality, as estimated from untreated subjects in toxicological work, and he provides a table which leaves no excuse for adopting more rough and ready adjustments on the plea that the labour of computation would otherwise be too heavy.

He next proceeds to point out that the probit method is used in investigation as well as assay work, and that in investigations several factors may, and indeed should, be varied at once. In such factorial experiments the response must be related to variation in all of these several factors, and allowance may have to be made for their interaction. The method of achieving these ends is illustrated by a case where three factors were varied, though one occurred at only two levels. Once the principle of this analysis has been grasped, there should be little difficulty in extending it to still more complex cases.

Turning to the behaviour of mixtures of poisons, the relations in action of the poisons are classified into three types. The data are available for a detailed study of the methods appropriate to two of these, similar and synergistic actions, but the discussion of independent action is of necessity less factual. It is in fact clear that more experimental data are required before certain aspects of the treatment of mixtures can be pushed much further statistically.

The general treatment is then concluded by a discussion of some special questions and formulae, and by the description of an ingenious application of the probit method to non-quantal, or graded, responses.

It will be seen that *Probit Analysis* is indeed a comprehensive, as well as an authoritative, treatment of its subject; and as such it is to be highly recommended. Its value is increased and its field of interest widened by its two Appendices and seven Tables. The first Appendix shows how the calculation and checking of a probit analysis can be reduced to a foolproof code, while the second extends the interest of the book in the other direction by developing in some detail the derivation of the method of analysis from Fisher's principle of maximum likelihood. Of the Tables nothing more need be said than that they form the most comprehensive collection of probit tables yet published, and that they will be indispensable to all who have probit analyses to do.

KENNETH MATHER

Leguminous Forage Plants. By D. H. ROBINSON. 2nd ed. Pp. vii + 119, with 35 Text-figures. London: Edward Arnold and Co. 1947. 6*s*.

Botanists and especially agriculturists will welcome the second edition of Dr Robinson's book on *Leguminous Forage Plants*. There are only a few books in English which deal as this one does with the botanical as well as the agricultural aspects of forage legumes. The style is clear and concise and the subject-matter is conveniently dealt with under several well-chosen headings. Students, advisers, and farmers will find the book invaluable in the identification and application of these crop plants. The general morphology of the seedling and adult stages

is simply, but accurately, described for each species. Other sections deal adequately, without being extensive, with types, strains, uses, seed production and chemical composition of each crop considered. Most users will appreciate the short contributions on the history of the species. In addition, the book contains a table giving statistics relating to size, weight and standards of purity and germination of the seeds of all the legumes described. The illustrations are very good and will be found exceedingly useful for aiding in identification, but a simple key to the species would have been a valuable addition in this connexion. Finally, the book is provided with a glossary of scientific terms. It can be thoroughly recommended for use by those interested in either the pure or applied aspects of the subject.

WATKIN WILLIAMS

Cytologie Végétale et Cytologie Générale. Par PIERRE DANGEARD. *Encyclopédie Biologique*, Paris. 1947. 1250 fr.

Cytology in Germany, Sweden, U.S.A. and Britain has grown up in close union with genetics. In all these countries it has become a practical basis of research in agriculture and medicine. In France, however, where there has been no genetics, cytology has existed as an independent science. How it has existed in these circumstances has been shown by the many publications of Guilliermond, Dangeard and their pupils. This is the latest. It seems to have been compiled from notes accumulated by Prof. Dangeard over more than 40 years and condensed into a mere 300,000 words. Its illustrations cover the whole of that period. Like the text they are indifferently representative of every aspect of the subject, the best and the worst. The book is therefore a mixture rather than a compound.

As a method of studying the history of those parts of the subject which are usually forgotten this book might be recommended. Dangeard's account of the cytoplasm, its containing wall and its constituent mitochondria and Golgi bodies is probably the best, and certainly the most extensive, that is likely to be available for many years. This account, however, is split in half by remarks on the nucleus whose study Prof. Dangeard says he finds difficult. His readers will find it even more so, and with better reason. For the author's difficulties are increased by his own invention as much as by the inherent magnitude of the problem. Perhaps one example will suffice to show his position. Having baptized the assemblage of vacuoles in a cell a *vacuome*, he endeavours to give them equal moment to the nucleus by naming the assemblage of nuclei in a cell or even in an organism a *nucleome*. This term should remain as a mystical monument to Prof. Dangeard's intellectual ingenuity and practical achievement, even when the details of his book have passed from memory.

C. D. DARLINGTON

German-English Science Dictionary for Students in Chemistry, Physics, Biology, Agriculture and Related Sciences. By L. DE VRIES. 2nd ed. Pp. xiv+558. London: McGraw-Hill Book Co. 1946. 22*s.* 6*d.*

In the review (*Ann. appl. Biol.* **28**, 407) of the first edition, this dictionary was ranked among the best of its kind. The new edition has 3000 more entries, giving a larger share to chemistry, but substantially increasing the number of words and idioms in all fields of science. As supplementary aids, there are lists of abbreviations, geographical names, conversion tables, chemical, electrical and thermodynamic symbols, and abbreviations of names of scientific periodicals. (Here it would be salutary to include also the correct abbreviations or contractions, as given in the *World List*.)

The volume is strongly bound in limp covers, clearly printed on good paper, and is small enough to rest comfortably in the palm. In form, as in content, it proves indeed a most serviceable handbook for the translator.

R. W. MARSH

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1946 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.
To Estimated Value of Stock, 1 January 1946	97	10	0	By Sales—Current Volume	1077	1	6
To Cambridge University Press	1969	17	10	By Sales—Back Volumes, Parts and Sets	347	1	5
				By Sales of Reprints and Adverts	87	12	0
				By Sale of Electros	3	15	0
				By Estimated Value of Stock, 31 December 1946	96	9	0
				By Balance, carried down	455	8	11
	£2067	7	10		£2067	7	10

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1946 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.	£	s.	d.
To *Annals of Applied Biology*, balance brought down	455	8	11	By Members' Subscriptions:						
To Printing and Stationery	21	15	5	Arrears	16	5	0			
To Postages and Cheque Stamps	23	3	0	Entrance Fees	25	14	6			
To Subscription—Parliamentary Science Committee	10	10	0	Current	491	5	0			
To Donation—The Biological Council	5	0	0					533	4	6
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer	17	14	3	By Sundry Income				1	12	7
To Expenses of Plant Pests and Diseases Committee	1	7	10	By Interest Receivable:						
To Audit Fee Reserve	5	5	0	National Savings Certificates	20	0	6			
To Balance, being Excess of Income over Expenditure for the Year	37	17	10	Post Office Savings Bank Account	23	4	8			
								43	5	2
	£578	2	3					£578	2	3

BALANCE SHEET, 31 DECEMBER 1946

Liabilities and Surplus	£	s.	d.	£	s.	d.	Assets	£	s.	d.	£	s.	d.
Sundry Creditors:							Cash at Bank:						
Cambridge University Press	479	7	11				Current Account	247	5	5			
Audit Fee Reserve	5	5	0				Post Office Savings Bank Account	1170	2	9			
Sundry Expenses	24	8	3								1417	8	2
				509	1	2	500 National Savings Certificates				775	14	10
Subscriptions and Entrance Fees, paid in advance				23	17	6	Stock of *Annals of Applied Biology* at estimated value				96	9	0
Life Membership Fund Reserve				25	0	0							
Excess of Assets over Liabilities:													
As per Balance Sheet of 31 December 1945	1518	15	6										
Add: Rockefeller Foundation Grant (per Royal Society)	175	0	0										
Balance of Income and Expenditure Account for 1946	37	17	10										
				1731	13	4							
				£2289	12	0					£2289	12	0

W. C. MOORE, *Hon. Treasurer*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed) H. J. COX & CO. *Incorporated Accountants* *Auditors*

HARPENDEN, 15 *May* 1947